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TRANSMISSION STUDIES OF MAIZE STREAK DISEASE[1]

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(With Plate I and 6 Text-figures.)

CONTENTS.

INTRODUCTION.

ALTHOUGH many methods of artificial transmission have been tried, streak disease of maize has been successfully transferred only by the agency of the leafhopper, *Balclutha mbila* Naude[2] (11). This apparent

[1] The matter of this paper formed in part a dissertation presented to the University of Cambridge for the degree of Doctor of Philosophy.

[2] I am informed by Mr W. E. China that this species properly falls into his new genus *Cicadulina* (*Bull. Entom. Research*, XVII, p. 43). Pending publication to this effect, however, it would appear that confusion will be avoided by retaining in this paper the name under which this species was originally described.

obligate relationship between the virus and a single species of insect causes the investigation of this disease to be a matter of particular interest. The studies described in this paper have been directed towards an understanding of the process of insect transmission of this disease; but, while they have thrown light upon certain aspects of the manner of action of the virus in the plant and in the insect, they are to be regarded as no more than a preliminary reconnaissance of a comparatively new field.

Work of a similar nature upon leafhopper transmission has been carried out by certain American workers with the curly leaf disease of sugar beet (2, 3, 4, 7, 8, 10); and the inspiration obtained from their papers is freely acknowledged. An account of an investigation by Kunkel (5) of aster yellows transmission has appeared since the work here reported was completed.

Acknowledgment is made to Dr I. B. Pole Evans, C.M.G., in whose Division I have worked; to Inspector J. S. Mackay and C. E. Levett, and in particular to Mr R. F. W. Nichols, for assistance in the manipulation of experiments.

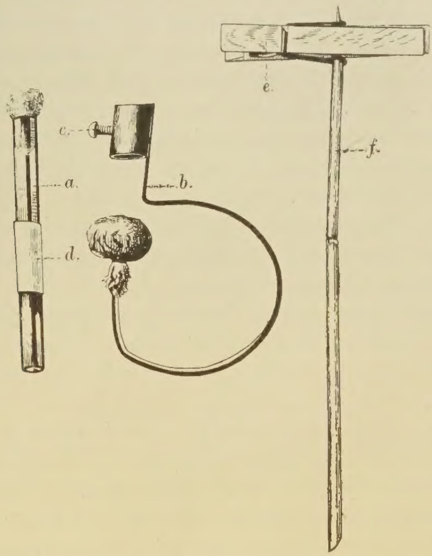
Methods.

The general principle of the experimental method adopted in these studies has been to confine the insects in small cages so that their feeding is localised to single leaves of plants, which are otherwise allowed to grow freely, unaffected by a general insect infestation and exposed to identical conditions as the controls. For this purpose it was necessary to develop a suitable form of leaf-cage and to have conditions under which the plants could be grown protected from the feeding of extraneous insects.

The glass-tube form of leaf-cage has been sufficiently described in my previous paper (11). The greater part of this work was carried out by an improved method. Glass tubes of approximately 6 mm. internal diameter and 8 cm. length, were held in clips in a manner which caused one end, ground square and smooth, to be pressed against the surface of the leaf. The spring-clip was constructed from spring steel (a kind of bicycle trouser-clip constituted the original material), soldered to a short length of brass tubing, and the whole was supported by a simple device made from a wooden clothes-peg and a piece of bamboo (Text-fig. 1).

The hoppers were manipulated into these tubes by making use of their tendency to move towards a source of light; but the pipette

method used by Kunkel (5) and others would have simplified this operation. The confined space of these small tubes did not provide conditions which *Balclutha mbila* was unable to tolerate, provided each tube contained a single hopper only and was moved every few days to a new section of leaf. In one experiment, in which a change was made to a new plant daily, a hopper lived for 116 days in one tube. The advantages of this technique over the large tube method were: greater rapidity



Text-fig. 1. The small tube technique used for transmission experiments with *Balclutha mbila*, showing glass tube (*a*) in which insect is confined; the spring-clip (*b*), in which the tube is held by the screw (*c*) bedding against a ring of rubber adhesive plaster (*d*); and the supporting stand, made from a spring clothes-peg (*e*) and piece of bamboo (*f*), passing through the middle of the spring.

of manipulation, a control of the point of feeding of the insect, and facility for the infection of very young seedlings, with a consequent reduction (as will be shown) of the incubation period of the disease in the plant (Plate I, fig. 1).

For protection from the visits of extraneous insects, the experimental plants were raised from seed within compartments of a greenhouse with gauze-protected windows. Although the house was old and in poor repair, a near approximation to insect-free conditions was

maintained. It was found however to be impossible to keep out red spider entirely; rarely also aphides made an appearance and occasionally escapes of *Balclutha mbila* occurred from the experimental cages. These difficulties were met by a frequent change-over from one chamber to another; the old chamber was emptied and cleaned as soon as the current experiments allowed, while all plants used in the new chamber had been raised from seed in that chamber. It was considered to be inadvisable to fumigate the house, since this procedure was liable to give a false sense of security; on the contrary, any insects which gained entrance were allowed full opportunity to develop and make their presence evident, so that the reliability of the experiments in progress might be fairly gauged. The records of the uninoculated control plants exposed in these chambers demonstrate that the experiments were carried out under conditions of nearly complete freedom from any significant insect infestation.

The experimental plants were Hickory King maize seedlings[1] grown about six to a tin, in a normal greenhouse compost. Usually the seedlings were infected while in the stage of showing one to three leaves (Plate I, fig. 1). The plants grew well and healthily, although the necessity for shading caused them to be somewhat drawn. They were however of good colour and always, during the period of observation, continued in good growth.

The breeding of the leafhoppers for experiment was carried out on maize leaf-tips in the large glass tubes, in which satisfactory development was obtained, except at the hottest period of the summer. The method of obtaining the originally uninfective parents was described elsewhere (11), and from eggs laid by their progeny uninfected cultures have since been maintained. The tube cultures required little attention, except that once or twice during their life it was necessary to transfer the young hoppers to clean tubes and fresh leaves.

In the early work the leafhoppers reared in this way were specially tested for infectivity. But since each "brood" was reared from the egg upon a leaf or leaves of a single healthy growing maize plant, this plant itself constituted an automatic control of the uninfective state of the hoppers. If this food-plant were still healthy when the hoppers reached maturity they were clearly uninfective.

In the course of the experiments here described, for the normal test of the infective power of individual leafhoppers a period of feeding of about seven days was allowed. My earlier work (11) showed that a single

[1] Seed supplied by the Principal, School of Agriculture, Cedara, Natal.

test of this duration afforded a reliable indication of the infective power of an insect, only one failure being there recorded in 47 tests of known infective individuals.

Controls.

Throughout this investigation, I followed the practice of marking as controls maize seedlings from the same sowings and generally growing in the same tins as those experimentally infected. These plants were retained under observation for the same period as the experimental plants. This account of my experiments is not burdened with detailed records of all the controls; but no experiment, except one (p. 9), has been included unless all its controls remained healthy. During the period January to October 1925 (when the majority of the results here presented were obtained), out of 1500 marked control plants five became diseased; meanwhile 1200 out of 3000 plants in a variety of experiments became diseased as a result of experimental infection by hoppers. Since controls and experimental plants grew within a few inches of one another, under identical conditions, these figures gave a strong assurance that the positive results obtained were not due to any cause other than that under study.

The virus in the insect.

Non-transmission of infective power through the egg.

Throughout my studies of *Balclutha mbila*, no evidence has been forthcoming to show that the virus of streak may pass from parent to offspring through the egg. The progeny of infective parents has always been uninfective, provided it has not had access to any source of infection.

This experiment has been frequently performed. For example, the progeny of infective parents was reared upon an immune variety of sugar-cane (P.O.J. 36) from eggs laid in a leaf of that plant; 36 adults, so reared were tested in a group successively upon two maize seedlings, which remained healthy. In another experiment, 24 hoppers, hatched from eggs laid in a fully green leaf of a streak-diseased plant (the leaf through which the plant had been originally infected by the parent hoppers) and removed within three days, proved to be all uninfective.

This failure of transmission through the egg is in accord with similar studies on *Eutettix tenella* (Stahl and Carsner(10)), *Cicadula sexnotata* (Kunkel(5)) and *Aphis rubiphila* (F. T. Smith(9)).

The acquisition of infective power by the different stages.

Experiments have shown that the young leafhoppers, as soon as they begin feeding upon a fully diseased leaf, may acquire the power of infection. The hoppers, hatching from eggs laid in a diseased leaf, were removed within 24 hours and tested singly upon maize seedlings. Four out of twenty caused streak infections. Two others, removed after 48 hours (but still in the first instar), both produced infections.

In the following experiment, summarised in Table I, young hoppers were removed from a culture on a streaked leaf and tested singly upon maize seedlings. When each hopper reached the adult stage, its sex was determined and the stage of removal calculated from the number of cast skins found. The results indicated that all instars and both sexes might be carrying infection.

Table I.

The acquisition of infective power by immature stages of Balclutha mbila.

Stage of development when removed from diseased leaf	Sex	Number tested	Number infective
2nd instar	Males	2	2
	Females	2	2
3rd ,,	Males	1	1
	Females	4	3
4th ,,	Males	2	Nil
	Females	2	2
5th ,,	Males	2	1
	Females	2	2

The young of *Balclutha mbila* occur as two types, some remaining a pale yellow until the final moult, while others assume a brown dorsal saddle-like mark after the second or third moult. This difference is not related to the sex of the insect. In the preceding experiment, both types of young caused infections.

Infective power was apparently not lost during the process of moulting. A culture upon diseased maize was kept under close observation, and, as adults emerged from the old skin in the final moult, before they had any opportunity of feeding, they were removed singly to healthy seedlings. Two out of five caused infections.

Evidence has already been presented (11) to show that adult leafhoppers of this species may develop the power of infection after feeding for a few days upon a streak-diseased plant. In five separate experi-

ments there described, in which the feeding period varied from 5 to 15 days, 26 per cent. of the males and 86 per cent. of the females became infective.

When the feeding period was reduced even to one hour a considerable proportion of the hoppers acquired the power of infection. In an experiment, carried out at an average temperature of 24° C., of 11 adult males, which fed for one hour on a diseased plant, 6 were afterwards able to cause infections when tested singly upon healthy maize plants. In a similar experiment at 30° C., 8 out of 26 adult females became infective.

Table II.

The proportion of leafhoppers infective after feeding throughout life on streak-diseased maize.

	Male hoppers			Female hoppers		
Date	Number tested	Number infective	Percentage infective	Number tested	Number infective	Percentage infective
Various	22	16	73	35	29	83
12. iii. 25	5	5	100	14	11	79
13. vii. 25	23	18	78	22	21	95
29. ix. 25	18	5	28	17	14	82
16. xi. 25	26	9	35	18	15	83
Totals	94	53	56	106	90	85

It rarely happened however that all the leafhoppers in any experiment became infective, whatever the duration of their feeding upon a diseased plant. In Table II are presented the results of a series of experiments, in which the leafhoppers lived upon the fully diseased leaves of streaked maize plants throughout the period from hatching from the egg until after the final moult. Of a total of 200 hoppers tested, only 143 were infective. The infective power was unevenly divided between the sexes and was found more frequently in the females, of which 85 per cent. were infective, as compared with 56 per cent. of the males. The females furthermore showed less irregularity as between separate experiments. It will be noted that the proportion of females which became infective after this long period of feeding was no greater than that obtained after a few days' feeding.

The occurrence of leafhoppers resistant to the acquisition of infective power.

The evidence of the preceding section points to the occurrence of individuals of *Balclutha mbila* which have some definite resistance to the acquisition of infective power. The belief that such resistant individuals occurred was stated in my previous paper (11), and of the cases there mentioned the following may be quoted in more detail, as an example of the manner of experimental study which has been followed.

Adult female Balclutha mbila, *collected on streaked maize*, 4. v. 24.
Tested twice upon healthy maize seedlings without producing streak.
Fed on streak-diseased sugar-cane for five days.
Tested three times on healthy maize seedlings without producing streak.
Fed on streak-diseased maize for nine days.
Tested on a healthy maize seedling without producing streak.
Died 8. ix. 24.

This hopper lived therefore for four months and failed to infect any of the six maize plants upon which it fed. In a similar way, six other uninfective individuals, which had fed upon diseased maize either in the field or under experimental conditions, were repeatedly tested, and proved, after one or more further periods of feeding on diseased maize, to be similarly resistant to the acquisition of infective power. In addition, certain of the uninfective individuals from the foregoing experimental tests of hoppers bred on streaked maize were re-tested after a second, and, in a few cases, third period of feeding upon streaked maize. Of eight such individuals, six resisted attempts to make them infective, while two became infective.

There can therefore be no doubt of the existence of a resistance in certain individuals of *Balclutha mbila* to the acquisition of streak infective power, and, although exceptions have occurred, it appears that this resistance cannot generally be overcome by further periods of feeding on a diseased plant.

In his studies of aster yellows, Kunkel (5) looked for, but failed to find, individuals of *Cicadula sexnotata* Fall. which were incapable of taking up the virus. McClintock and Smith (6), on the other hand, were of the opinion that only 50–60 per cent. of their aphides were vectors of spinach blight.

The progeny of resistant females of *Balclutha mbila* may become infective. Thus 14 adults were reared from eggs laid by the resistant

female considered on p. 8, and after feeding for nine days on a diseased leaf, seven out of the fourteen were proved to be infective.

The retention by leafhoppers of the power of infection.

In my early work upon *Balclutha mbila* (11), all the hoppers studied appeared to retain the power of infection until death; one individual, which lived for 150 days, infected a maize plant during the last ten days of its life. This aspect of the problem has been further studied by transferring single hoppers daily to new healthy plants. Three series of experiments[1] were carried out, as follows:

MALES:	No. 1.	Lived 84 days.	Infected 47 plants.
	„ 2.	„ 116 „	„ 58 „
	„ 3.	„ 49 „	„ 32 „
FEMALES. *Series A:*			
	No. 1.	Lived 22 days.	Infected 21 plants.
	„ 2.	„ 26 „	„ 23 „
	„ 3.	„ 26 „	„ 26 „
Series B:			
	No. 1.	Lived 111 days.	Infected 19 plants.
	„ 2*.	„ 111 „	„ 63 „

(* accidentally killed)

With the exception of the one individual, No. *B* 1, the hoppers showed no indication of a loss of infective power. Thus, for example, No. *B* 2 infected 18 out of the first 30 plants and 19 out of the last 30. But with No. *B* 1 the contrary applied; after infecting 16 out of the first 33 plants, this hopper appeared to show a progressive weakening of infective power, infecting plants thereafter only on the 42nd, 45th and 66th day, and failing to infect any plant during the last 45 days of its life.

Whereas in my early studies, leafhoppers, once proved to be infective, very rarely failed to infect any plant upon which they fed for about 7 days; the preceding experiment demonstrates that frequent failures may occur when the feeding period is a short one. The longest periods of consecutive daily infections were 26 days in the *A* series females, and 11 days in the other series; while the longest period of consecutive failures was 6 days (excluding No. *B* 1). But, on the whole, the distribution of the positive and negative results seemed to be quite

[1] Two control plants in these experiments became diseased.

irregular, and afforded no support to the idea that the insect might undergo a recurring cycle of infective and uninfective periods, therein agreeing with the conclusions reached for *Eutettix tenella* by Severin (8) and Carsner and Stahl (4).

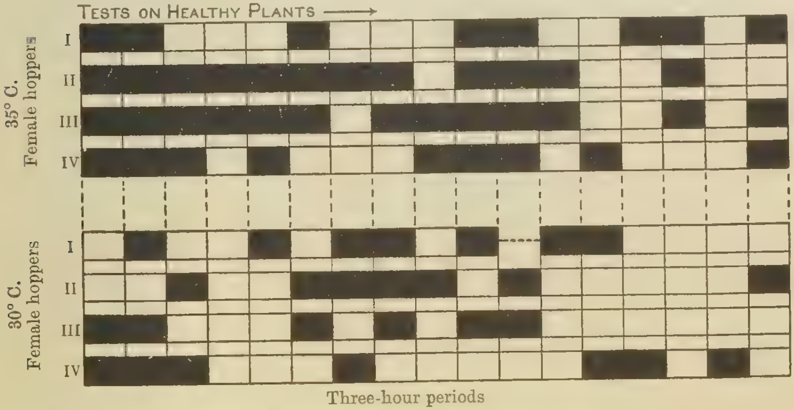
A comparison of these experimental results with the temperature records of the greenhouse indicates that the higher temperatures (up to a maximum of 31° C.) somewhat favoured infection. Furthermore, the experimental plants, which were not of uniform age, appeared to show an increasing resistance with age (see p. 17).

Further experiments were performed in which the effects of varying temperature and age of plants were eliminated. The leafhoppers were kept continuously in the dark in incubators, the plants being transferred from the greenhouse to the incubators only for the infection-period. By daily sowings a supply of plants was maintained of a uniform age at the beginning of each infection-period. In an experiment at 30° C. one adult female, moved to a new plant every 24 hours, infected eight plants out of nine, while a second infected eight out of eleven. The feeding period was reduced to three hours in a second experiment, the results of which are presented graphically in Text-fig. 2[1]. At 35° C. four adult females, tested for 17 consecutive three-hour periods, infected 42 out of 68 plants, while in a similar experiment at 30° C. 27 infections were caused in 67 plants (one plant being lost). These results again afforded no indication of a cycle of development of the virus in this leafhopper.

In all these experiments the insects appeared to be feeding upon the plants, although observation alone cannot give certainty on this point. But when leafhoppers were starved at 30° C., the females began to die after 12 hours, and 70 per cent. were dead in 24 hours. It is highly improbable therefore that the failures to obtain infections in 24-hour periods were due to a failure of the insects to feed. If, however, the irregularity of my results were due to a periodical disinclination to feed,

[1] In this diagram, and in Text-figs. 3 and 4, the history of an individual hopper is to be followed in each horizontal column. The period of feeding of the hopper is indicated by the vertical lines; at the intersection of every vertical line with the horizontal column the hopper was moved on to a fresh plant. The resulting small rectangles are black, if, as a result of the feeding of the hopper during that period, the plant became streak-diseased; and white, if infection failed. Where, in Text-figs. 3 and 4, the rectangle is cross-hatched, the hopper fed upon a fully diseased maize leaf during that period. A break in the horizontal column, joined by a dotted line, denotes the loss of the record for that period. The circles, in Text-figs. 3 and 4, show whether infection resulted in plants on which the hoppers fed for several days at the conclusion of the experiment proper.

then this disinclination would be overcome by preliminary periods of starvation. The results of an experiment carried out at 30° C. are presented in Table III. The hoppers were taken directly from a culture upon a streaked plant, and were not individually proved to be infective beforehand; when any hopper died it was replaced by another from the same original culture, which had undergone the same starvation period.



Text-fig. 2. Diagram showing the infections of maize seedlings resulting from the feeding of single infective leafhoppers for successive three-hour periods at 35° C. and 30° C. (See footnote, p. 10, for explanation.)

This experiment demonstrated that preliminary starvation had no effect in increasing the number of infections caused by individual hoppers in three-hour periods. Incidentally it was again shown that considerable periods of fasting are abnormal for this leafhopper; for, whereas in the early part of the experiment no hoppers died, during the three-hour starvation periods four hoppers died and were replaced, while during the six-hour starvation periods ten hoppers died.

Table III.

The influence of preliminary starving upon transmission by individual leafhoppers.

Nature of infection period	No. of tests	No. of infections	Percentage of positives
3-hour periods without preliminary starvation	48	32	67
3-hour periods after 3 hours' starvation	47	21	45
3-hour periods after 6 hours' starvation	44	26	59

Experiments have been performed to determine whether infective power is retained during periods of feeding upon resistant plants. The fact that I was able to breed on such plants uninfective hoppers from eggs laid in them by infective parents indicates that these plants were not acting as symptomless carriers of streak. Two female adults of *Balclutha mbila*, having been proved to be infective to maize, fed for 29 days on sugar-cane (variety Uba). Each hopper was then tested twice upon healthy maize seedlings, which became streaked. After feeding for a further 84 days on cane, the hoppers were shown still to be infective to maize. The cane plants remained healthy during observation periods extending over two and five months respectively.

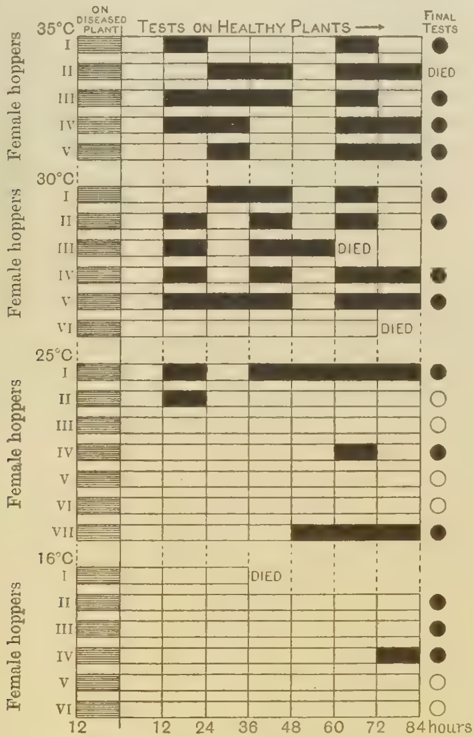
It appears therefore from these several results that while the infective power is usually not lost by *Balclutha mbila*, such loss may occasionally occur. In the case of *Eutettix tenella* such a loss was reported by Boncquet and Stahl (2) to occur in 15–35 days, but denied by Severin (8). Carsner kept infective hoppers on apparently immune plants for 58 and 111 days without loss of infective power (3). Kunkel (5) found that, while some individuals of *Cicadula sexnotata* retained infective power through life, others lost it quickly.

A delay in the development of infective power.

The studies of the preceding section, carried out for the most part with leafhoppers which had, as a preliminary step, been proved to be capable of infecting maize plants with streak disease, afforded a guide as to the frequency of the infections which might be expected to occur when the period of exposure of the plant to the insect's feeding was short. By following the procedure of moving a leafhopper to fresh healthy plants after short intervals of time, it was possible therefore to decide whether any significant uninfective period intervened between the insect's feeding upon a diseased plant and the development of infective power.

Preliminary experiments were carried out in the greenhouse, and in each case were duplicated by a series starting after a 12 hours' delay, in order to compensate for any possible influence of night and day conditions. Adult uninfective female hoppers fed first for 12 hours upon a diseased leaf and thereafter were transferred after each 12-hour interval to healthy maize seedlings. At the conclusion of the experiment the hoppers were tested finally for several days upon healthy plants.

In two such experiments, eight hoppers out of nineteen failed altogether to become infective. Of the remainder, one produced its first infection in the first period upon a healthy plant, two in the second period, one in the third, three in the fourth, one in the sixth, and one only in the final test after it had failed to cause any infections during

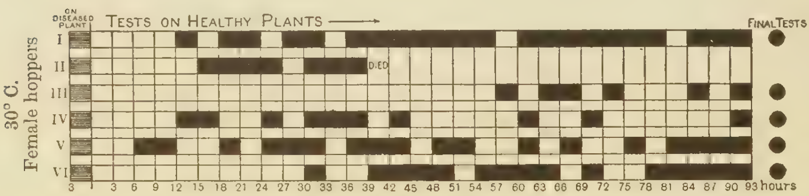


Text-fig. 3. The incubation period of the virus in the insect. Diagram showing the infections of maize seedlings caused in successive twelve-hour periods by the feeding of originally uninfective leafhoppers which had fed for twelve hours upon a diseased plant. At temperatures of 35° C., 30° C., 25° C. and 16° C.

six periods. In each of these cases the hopper, after causing its first infection, continued to infect a large proportion of the plants in subsequent periods; in all 37 out of 51 plants, subsequent to the first infection, were infected. In addition two anomalous cases occurred in which two hoppers infected single plants during the seventh and ninth

periods respectively, but none thereafter (including the final test plants). While this result suggests accidental infections, this is improbable, since a full series of control plants remained healthy. The temperature during these experiments averaged about 22° C., but showed great daily fluctuations.

A similar procedure was followed in a series of experiments at constant temperatures, the results of which appear in Text-fig. 3. An absence of infections during the first 12-hour period was encountered uniformly throughout the whole series. At 35° C. and 30° C. the majority of the hoppers developed infective power during the second period or less frequently during the third period. At 25° C. two hoppers infected during the second period, while two others showed a delay to the fifth and sixth periods respectively. The results at 16° C., while indicating



Text-fig. 4. The incubation period of the virus in the insect. Diagram showing the infections of maize seedlings caused in successive three-hour periods by the feeding of originally uninfective hoppers which had fed for three hours upon a diseased plant. At 30° C.

a lengthy uninfective period, are to be accepted with caution, since the normal frequency of infection at this temperature has not been studied. One anomalous case occurred at 25° C. where a hopper infected in the second period but failed thereafter.

This experiment was repeated with the feeding periods on each plant reduced to three hours. The results of this series, presented in Text-fig. 4, show the first infections occurring in one case in the third three-hour period and, at the other extreme, one only in the twentieth period; that is, after the hopper had caused no infections for 57 hours. Again, each hopper, after it had produced its first infection, continued to infect a considerable proportion of the plants to which it was subsequently moved.

In the light of these observations, it can hardly be doubted that a significant delay occurs in the development of infective power in this

leafhopper. In one case only, out of a total of 34, did streak disease result in the healthy plant to which a hopper was first transferred after its short feed upon a diseased plant.

On the other hand, the great irregularity in the behaviour of individual leafhoppers makes impossible any exact numerical expression of the duration of this uninfective period. It is clear, however, that at temperatures of 30° C. to 35° C., this period lies usually between 12 and 48 hours. The absolute minimum observed was a period of between 6 and 12 hours at 30° C. In the controlled temperature series, the maxima observed were 57–63 hours at 30° C., 60–84 hours at 25° C. and $>$ 84 hours at 16° C. (the last somewhat unreliable).

These results are comparable with those obtained for *Eutettix tenella* (4, 7) where a minimum uninfective period of four hours was observed at 100° F. (37·8° C.). Kunkel (5), however, reported an incubation period of aster yellows virus in *Cicadula sexnotata* of more than ten days.

Comparison of the effects of single hoppers and of several hoppers together.

Since the majority of the results presented in this paper have been obtained by the study of individual leafhoppers, it is well that a comparison should be made with the effect of groups of hoppers.

In an experiment young maize seedlings were subjected to the feeding for 24 hours of single hoppers and of groups of four hoppers, all from cultures on streaked maize. The grouped hoppers caused 15 infections in 24 plants, the single hoppers 12 infections in 48 plants. On the average, the disease appeared more quickly in the "group" series of plants; but in each series, the shortest individual incubation period observed was the same—three days to the first spots and six days to the full development of streak.

This experiment was repeated with groups of 12 hoppers. While single hoppers infected 7 out of 24 plants, the groups infected 23 out of 24. Again the average incubation period was shorter in the "group" series, although there was no difference in the minimum incubation period in each series.

Throughout both experiments the severity of the disease, where infection occurred, was approximately the same in every plant.

It would appear therefore that the effect of a group of hoppers is the effect of the most "powerful" individual of the group, and is not the average effect of the members of the group, nor the sum of the

effects of the members. This is in general agreement with the conclusion reached by Carsner and Stahl (4).

The virus in the plant.

The state of the plant in relation to infection.

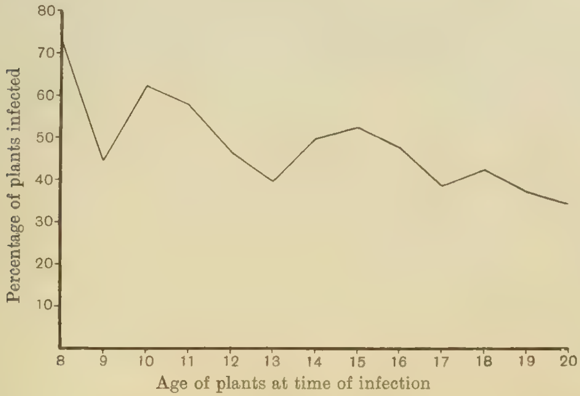
The studies described in the preceding section have demonstrated that the effect of the feeding of *Balclutha mbila* upon a healthy maize plant is dependent, on the one hand, upon the individuality of the leafhopper and its previous history. It is plain however that, in that critical process which may lead to the development of streak disease, the individuality of the plant and its environment must also play a large part. In this section are summarised certain preliminary studies of the plant in relation to infection.

It would be expected that the reaction of the plant would be made evident mainly in two ways: by some resistance to infection, preventing any development of the disease, and by variation in the duration of the period of incubation of the disease in the plant. While the observations of the former of these presents no difficulty in experiment, the incubation period is a quantity of considerable uncertainty. I have usually adopted the period between the beginning of exposure to infection and the first appearance of the very characteristic first spots of streak (see (11), Plate XVI, fig. 3). But after this first appearance there is often great variation in the time taken before the full onset of the disease. Severin's (7) method of determining the incubation period by finding the stage at which hoppers could obtain the virus from the plant was, for reasons which will appear later, inapplicable to streak disease.

Opportunity has not permitted of a special search for hereditary factors for resistance in maize. I have been fortunate in these studies, in dealing with a plant of very low inherent resistance to streak disease, with the consequence that my experimental results have shown a regularity unusual in work of this type. Where, however, the inoculation has been small (that is, the period of the insect's feeding short) it is a matter of doubt whether the irregularity of infections so obtained may not have been due to a varying resistance in individual plants.

That the age of the plant at the time of exposure to infection has a great influence upon its reaction to the streak virus has been evident throughout all my experimental work. In the experiment in which hoppers were transferred daily to new plants (which in this case varied

between 8 and 20 days old from sowing) a distinctly lower proportion of infections occurred in the old plants than in the young (Text-fig. 5). The influence of the age of the plant upon the incubation period was experimentally tested; a number of plants, all of one day's sowing, were infected on successive days by groups of four hoppers feeding for 24 hours on the first leaf of each plant. In this way a series of plants, varying in age between 5 and 17 days from sowing, was subjected to infection by the same groups of insects. At ages up to 10 days from sowing the incubation period was nearly uniform and averaged 3·5 days; but in plants of greater age there was a tendency for the development



Text-fig. 5. The percentage of plants, of different ages, which became streak-diseased as the result of the feeding of individual leafhoppers for 24-hour periods. (Results of 471 trials in experiments described on p. 17.)

of the disease to occupy a longer period of time, the 17-day old plants showing the first spots only after an average of 7·5 days.

I have been unable, for lack of suitable equipment, to study the development of streak in plants at controlled temperatures; but the great influence of high temperatures in increasing the probability of infection and in shortening the incubation period has been obvious. Nor has it been possible to study the effects of humidity or of intensity or length of lighting, although I have no evidence to suggest any great influence exerted by these factors. While it is probable that the soil conditions may influence streak infection, an experiment upon soil moisture afforded no indication of this. Two series of plants were kept, the one watered very heavily and the other only lightly when necessary

to prevent the plants wilting. As a result of two days' feeding by single hoppers, 10 out of 12 "wet" plants became diseased and 8 out of 12 "dry" plants; the average incubation periods were 11·2 and 11·4 days respectively.

The point of inoculation.

The adoption of the small tube technique permitted an exact control of the point of feeding of the leafhopper, that is, the point of inoculation with the streak virus. Infections were obtained whether the insect fed upon the upper or lower surface of a leaf. In one experiment, in which single hoppers (taken from a culture which showed an exceptionally low proportion of infective individuals) fed alternately upon the upper and lower surfaces of separate plants, 9 out of 40 plants were infected from the upper surface and 11 out of 40 from the lower surface.

Infection occurred as readily through young as through old leaves. But an interesting result was obtained when the insect was placed upon the tip of the youngest leaf of a plant. Frequently, as this leaf grew out, it would bear streak symptoms at its base, therein proving an exception to the usual experience that the leaf upon which the hopper feeds shows no visible effects of that feeding (Plate I, fig. 2). It was observed that, in a plant infected in this manner, while the first spots might often appear within 48 hours, yet the full onset of the disease was often delayed considerably beyond the period which was normal when infection occurred through an old leaf. In a comparative experiment, a plant, infected through an old leaf, showed first symptoms on the fifth day and the full development on the tenth; whereas a similar plant infected through the youngest leaf had first spots on the third day but the full form of the disease only on the fifteenth.

The movement of the virus in the plant.

In that the effect of infection by the streak virus is first shown by the young developing terminal leaves, it must be supposed that, where inoculation has taken place at the tip of an old leaf, the virus must have passed down that leaf and into the stem of the plant. I have attempted to study this movement of the virus.

Infection is not prevented, nor its results delayed, by certain forms of leaf mutilation. This was demonstrated experimentally for the cutting out of the main leaf vein (Text-fig. 6 *a*) and the severing of the veins of half the lamina, as shown in Text-fig. 6 *b*. This result was to have been expected, for in either case the leaf remained turgid, being supplied by

the small anastomosing veins which form a network through the leaf lamina.

By an experimental procedure of completely severing the leaf at a definite time after the hopper began to feed upon it, it was possible to examine the rate of movement of the virus down the leaf. For if, in spite of severing the leaf, the plant became diseased, the virus must in the given time have passed beyond the point of cutting. While my investigations along this line are incomplete, and certain anomalous results are as yet unexplained, the following positive results of experiments, carried out at 30° C. with groups of 12 infective hoppers, may be now reported.

After one hour, leaf cut off 10 cm. below point of feeding—three infections resulted in eight plants.

After two hours, leaf cut off 40 cm. below point of feeding—three infections resulted in eight plants.

Text-fig. 6. Types of leaf mutilation employed in experiments on transmission. The dotted circle represents the position of the small tube containing the infective leafhopper. The midrib is cut out in *a*, and half the lamina in *b*.

This great speed of movement is comparable with the results obtained with the beet curly-leaf virus by Severin (8), whose method of procedure has been followed by me. The highest speed recorded by him was 7 in. in half an hour at a temperature of 103·5° F. (39·7° C.).

A number of experiments have been carried out to determine whether the virus in its passage down the "infection leaf" was available to be taken up by uninfective leafhoppers feeding upon that leaf; and also, whether the virus became likewise available in the other fully formed leaf tissue of the plant. These experiments are summarised in Table IV. It was found that a small proportion of the uninfective hoppers which fed upon the lower part of the infection leaf would become infective, particularly if the infection of the plant were caused by a large number of infective hoppers. On the other hand, I obtained no evidence that the virus passed up into the fully formed tissue of other leaves, except

only in the experiment No. V, which I was quite unable successfully to repeat. In the later experiments, uniform failures were encountered, even although the young leaves, upon the tips of which the uninfective hoppers were feeding, themselves developed full streak symptoms on their lower parts.

Table IV.

The presence of the virus in various parts of the maize plant during the development of streak disease.

No. of experiment	Part of plant fed upon by uninfective hoppers	Infecting agent (infective hoppers)	Period of feeding (days)	No. of hoppers tested	No. of hoppers which became infective
I	Lower portion of leaf through which infection is occurring	8	15	14	Nil
II	Same	30	14	18	8
III	Same	50	8	50	7
IV	Next leaf above infection leaf	8	28	9	Nil
V	Tip of youngest leaf, just appearing at start of experiment, which later developed streak symptoms on its lower part	6	10	21	2
VI	Same	30	14	15	Nil
VII	Same	60	7	40	Nil
VIII	Same	36	6	15	Nil

These results stand in notable contrast to those of Severin (7, 8), who easily infected hoppers from fully formed leaves of sugar beets, even before the plants had developed any visible symptoms.

The localisation of the virus in the diseased plant.

The leaves of a streak-diseased maize plant bear the almost completely chlorotic stripes, which are characteristic of this disease, separated by green areas, which appear to be normal (see (11), Plate XV, fig. 2). The oldest leaves, if still living, are devoid of chlorotic areas, or show these areas only in their basal part. In any of my experimentally infected plants, one of these leaves was known as that through which the virus has passed during infection. By feeding uninfective leafhoppers upon the several parts of a diseased plant, I have endeavoured to determine the location of the virus, or, at least, its location in a form available to the leafhoppers.

The virus may occasionally be obtained from the "infection leaf" of a fully infected plant. As has been already recorded, this never occurred when the hoppers, hatching from eggs laid in the "infection

leaf," were removed within a day or two from this plant. But when the period of feeding was considerably extended, a few hoppers became infective. Of 40 hoppers reared to maturity upon the "infection leaf," 4 were infective. In another experiment, 5 hoppers became infective out of 18 which fed on the "infection leaf" for 7–14 days after hatching.

Table V.

The presence of the virus in various parts of the streak-diseased maize plant.

Portion of plant upon which hopper fed	No. of hoppers tested	No. of hoppers infective
Entirely green leaf below all streaked leaves	6	Nil
Entirely green terminal portion of leaf bearing streaks towards its base	8	Nil
Green part of same leaf below the level of the highest spots of streak but above the point where the spots become fully crowded	7	1
Controls:		
Yellow areas on fully streaked leaf	26	21

The other green parts of a fully diseased plant gave almost entirely negative results. In a group of experiments, the results of which appear in Table V, the uninfective hoppers fed for seven days in small tubes clipped upon the several parts of a diseased plant. One hopper only out of 21 obtained the virus from the green part, whereas 21 out of 26 obtained it from the yellow part.

In order to test for the presence of the virus in the green areas separating the chlorotic stripes, a piece of tracing cloth was placed between the end of the small tube and the leaf, and adjusted so that a hole, about 2 mm. by 1 mm., cut in the cloth, exposed the required area of the leaf to the hopper's feeding. The technique was difficult, since the positions of the tubes frequently became altered and many hoppers, failing to find the holes, died of starvation. The results of seven separate experiments were as follows: of 32 hoppers fed upon the green part, 16 became infective, while of 17 fed upon the chlorotic part, 16 became infective.

This point was further investigated by cutting out the required portions from a diseased leaf, and exposing these small portions to the insects' feeding. This experiment was performed three times with the following aggregate results: of 26 hoppers fed on the green portions, 4 became infective, whereas of 22 fed on the yellow portions, 16 became

infective. These results undoubtedly denote some significant difference in the occurrence of the virus in the green and yellow areas. On the other hand, I am unwilling to accept all the positive infections from the green part as due to experimental error, although by the nature of the experiment this error was likely to be high.

In this apparently localised distribution of the virus streak disease stands in marked contrast to curly-leaf of beet, where the virus has been found to be present in the old, apparently normal, leaves (4, 8).

Discussion.

These studies were undertaken primarily with the purpose of obtaining an understanding of the many factors involved in the process of streak transmission by *Balclutha mbila.* Upon certain points they have provided a definite answer; and they have indicated some of the precautions to be observed, if the most nearly consistent results are to be obtained in experiment.

On the whole they have served to confirm, in the special case studied, many of the views now generally held concerning the insect transmission of virus diseases. Thus, it can hardly be questioned that some specific biological relationship exists between *Balclutha mbila* and the streak virus. The evidence clearly indicates a multiplication of the virus in the insect. While it may be argued that the insect in reality is merely becoming repeatedly reinfected by virus which has multiplied in the plant at the point of feeding, yet this is not a reasonable criticism, in view of the evidence presented of the permanence of the infective power of the leafhopper under different conditions. The incubation period in the insect admits of two explanations: either it is the time taken for the virus to move from the gut to the salivary glands and perhaps also to multiply up to the point where an infective dose passes out with the saliva; or, possibly the virus undergoes a cycle of development within the insect. It would be premature at this stage to postulate an obligate cycle, for it is not impossible that the leafhopper's success in transmission is due in part to some detail of the process of inoculation by means of the setae, which cannot be reproduced mechanically. In support of this view is the evidence that many of the viruses, even the most refractory, such as that of sugar beet curly-leaf (8), have been shown to infect by direct inoculation of plant juice, when a suitable technique has eventually been found. But, on the other hand, the limitation of transmission to the one species of leafhopper alone shows that there is probably more involved than mere facility in inoculation.

The occurrence of resistant individuals of *Balclutha mbila* suggests some form of protective mechanism against infection by the virus. These individuals can hardly have defective mouth parts, for they have lived and fed for periods up to four months. Dr T. J. Naude has examined such individuals and declared them to be typical *Balclutha mbila*, while the progeny of one resistant female was proved able to infect. Nor is it feasible that during their long periods of feeding on diseased plants, these individuals accidentally missed the yellow areas containing the virus.

For infection of a maize plant to occur it would seem that a minimal dose of virus is required, beyond which an excess produces no additional effect. It is clear from my results that (unless the period of inoculation be a long one) this minimal dose may often not be inoculated, although the insect be certainly feeding and be certainly carrying the virus. Conceivably infection will result only if the virus be inoculated into a particular tissue not always tapped by the insect in its feeding. If it be that the quantity of virus in the saliva of an individual hopper varies at different times, my experiments afford no indication of any rhythmic change in concentration. It is not impossible that the irregularity of many results may be due simply to varying resistance in individual plants, that is, to a varying appropriate minimal dose.

The localisation of the virus in the chlorotic areas of the diseased leaf appears to be an unusual feature in this group of diseases, although there is reason to believe that it will be found to be of more general occurrence in other cases, when the distribution is studied by a suitable technique. Conceivably streak disease offers a parallel to the infectious chlorosis of the *Malvaceae* of Baur (1), who considered that the yellow areas were acting as multiplying centres for the virus, whence it passed to the meristematic region. If this be the situation in streak disease, it is possible that the occasional leafhopper, which becomes infective after feeding upon the green part of the diseased leaf—if this be not a consequence of experimental error—may have intercepted the virus in its passage down from the multiplying centres, just as experiment has shown that it may be intercepted in its passage down from the point of inoculation.

Summary.

For the foregoing investigation of the relation between the virus of streak disease and, on the one hand, the insect-vector, *Balclutha mbila* Naude, and, on the other hand, the maize plant, a special technique

was developed in which the insects were manipulated and studied individually.

The eggs laid by infective leafhoppers of this species produced uninfective progeny, but the young leafhoppers might in any stage of development become infective after feeding upon a diseased maize plant. This infective power was retained during the change of skin. Experiments showed that adults might acquire the power of infection after feeding for one hour, but in lower proportion than was the case when the feeding period was of several days' duration. But even when the feeding period on a diseased plant extended through the whole course of development of the insect from the first instar to the adult stage, a proportion of the hoppers remained uninfective. Experimental study of these uninfective individuals led to the conclusion that no further periods of feeding on a diseased plant would make them infective. Nevertheless, the progeny of such resistant females might become infective.

A study of the infections produced by individual hoppers, when repeatedly transferred to fresh maize plants after short time intervals, gave no indication of a cycle of alternating infective and uninfective periods in the insect. The frequency of infection was increased by high temperatures, but was not influenced by preliminary starving of the hoppers.

The power of infection was usually retained through the life of the hopper, but one definite exception was observed.

Experiments showed that there occurred an incubation period of the virus in the insect, of variable duration but shortest at the higher temperatures. The minimum period observed was 6–12 hours at 30° C.

Comparative studies of infection by single hoppers and groups of hoppers showed that the groups caused infections more frequently and, on the average, in less time, but not more quickly than the quickest of the individuals feeding alone.

With regard to the state of the plant at the time of infection, it was found that the frequency of infection and the incubation period of the disease in the plant were affected by the temperature and the age of the plant, but apparently not by soil moisture. Infection occurred as frequently when the hoppers fed upon the upper or lower surface of the leaf and on the young or old leaves, although infection through a young leaf might cause the first symptoms to appear in an unusually short period.

The passage of the virus down a leaf inoculated near its tip was not

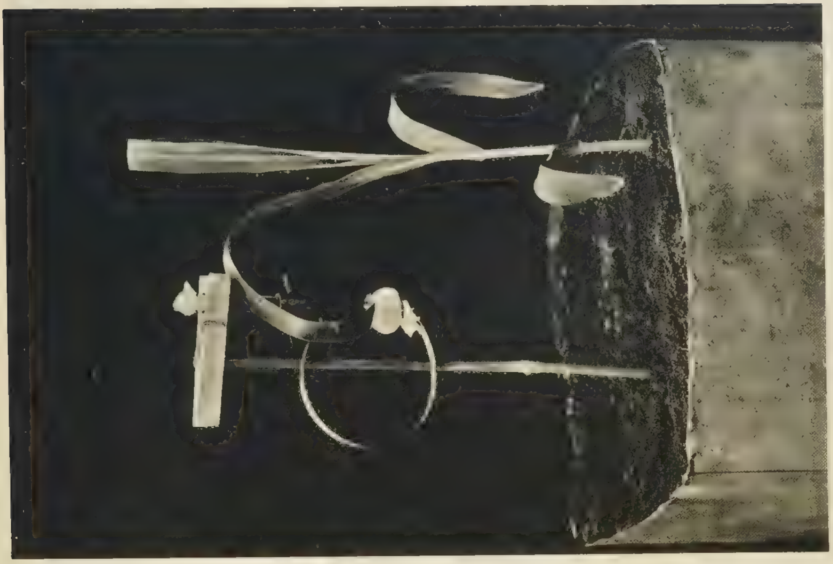


Fig. 2.

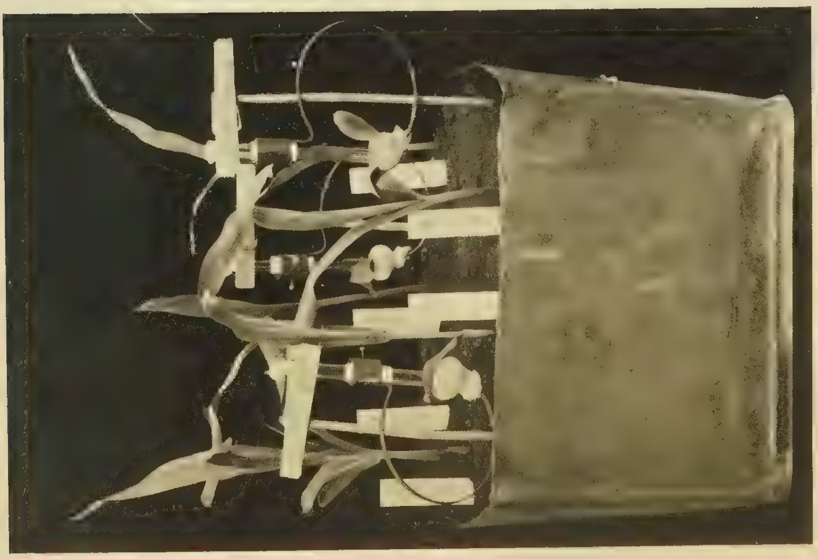


Fig. 1.

STOREY.—TRANSMISSION STUDIES OF MAIZE STREAK DISEASE (pp. 1–25).

affected by certain forms of leaf mutilation. This downward movement occurred at a rate exceeding 40 cm. in two hours.

From the diseased plant the virus was usually obtained by the leafhopper after feeding on the chlorotic areas but not from the green areas, except that a small proportion of hoppers usually became infective after feeding on the leaf through which infection took place, and similarly a small proportion (perhaps due to experimental error) after feeding on the green areas lying between the chlorotic areas in the fully diseased leaf.

The Natal Herbarium,
Durban, South Africa.

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EXPLANATION OF PLATE I.

Fig. 1. The small tube technique in use for a streak infection experiment. Each tube contains a single individual of *Balclutha mbila*, which is confined in its feeding to a small area of a single leaf of each maize seedling. The control plants are growing in the same tin of soil. $\times \frac{1}{4}$.

Fig. 2. Infection of a maize seedling through the tip of the youngest leaf. As the plant grows streak symptoms may appear towards the base of this leaf, as shown in this photograph.

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THE ROSETTE DISEASE OF PEANUTS (*ARACHIS HYPOGAEA* L.)

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(With Plates II–VI and 2 Text-figures.)

CONTENTS.

Introduction.

In several tropical and sub-tropical countries there has been recognised in recent years an abnormal condition of the ground-nut or peanut (*Arachis hypogaea* L.), characterised by stunting, chlorosis and sterility. While, hitherto, investigators have failed to associate this condition with any parasite or to produce it experimentally, Zimmermann's suggestion in 1907 (10), that it is a disease of the virus group, has come to be generally accepted by plant pathologists. Our studies, reported hereunder, in which we have experimentally transferred the South African

form of this disease by grafting and by insect-vectors, lend support to this suggestion.

This investigation was begun in 1923 by one of us (A. M. B.); for the work subsequent to 1924 the other author is mainly responsible. We record our thanks to Dr I. B. Pole Evans, C.M.G., Chief of the Division of Botany, under whose general supervision we have worked; to Field Inspector J. S. Mackay, whose work under our direction in the field and greenhouses led to the discovery of the insect-vector; to Mr R. F. W. Nichols for assistance in the manipulation of later experiments; to Messrs African Explosives and Industries Ltd., Umbogintwini, Natal, for facilities for field experiments; and finally to many farmers in the Waterberg district and in particular to Mr E. E. Galpin, F.L.S.

Occurrence.

Rosette disease has a wide distribution through the peanut-growing areas of the Union of South Africa. Our earliest record dates from 1909, but the disease assumed prominence only in 1922–23, when a severe outbreak administered an important check to the expanding peanut industry of the Waterberg district of the Northern Transvaal (3500–4000 ft. elevation), where in later years the area planted by each farmer has remained stationary or tended to decrease. In each subsequent season the disease has reappeared in that district with varying severity; and in the meanwhile its occurrence has also been noted in the districts of Rustenburg (4000–5000 ft.), Pretoria (4500 ft.), Pietersburg (2000–4000 ft.), Barberton (2500 ft.), and in the coastal belt of Natal and Zululand (to sea level).

We have little hesitation in identifying our rosette with the East African "krauselkrankheit" of Zimmermann (10, 11). His descriptions and illustrations indicate a condition very closely agreeing with the diseased peanut plants of the Union. Zimmermann recorded severe damage by this disease in years up to 1913 in the Lindi region and other parts of what is now Tanganyika Territory, where Kirby (4) reported the disease to be still serious in 1924. In West Africa, Bunting (1, 2) has recorded, without description, a "bunching" disease of ground nuts, known also, according to Mr A. H. Kirby (in correspondence), in Nigeria. Reports of a rosette-like disease have been received from Southern Rhodesia. These records suggest that the disease which we have been investigating is of wide distribution through tropical and sub-tropical Africa.

The identity of the Javan "krulziekte" (7) with our rosette is a less certain matter, although Rutgers considered the agreement with "krauselkrankheit" to be almost complete. But recent findings in virus disease research point to the danger of assuming the identity of two virus diseases bearing a superficial resemblance but occurring in widely separate geographical regions. Furthermore, the peanut plants in Rutgers' illustrations lack the extreme shortening of the petioles to which we are accustomed. In Java the disease was first noted in 1908 and sporadic outbreaks have been reported in subsequent years. A somewhat similar rosetted condition was found in Java in six other leguminous plants and four non-legumes (7, 8).

In correspondence, the Government Mycologist, Coimbatore (S. Sundararaman), has supplied us with a description of a "clump" disease of ground nuts in Madras, which indicates a condition closely similar to rosette. Nothing appears to have been added to McClintock's 1917 record (5) of mosaic in a single peanut plant in Virginia, U.S.A.

Description.

Rosette disease produces a striking modification in the peanut plant; the whole plant may be little more than a close tuft of small curled leaves, forming a cushion of a few inches diameter (Plate II, fig. 1), or its branches may be of some length, but bear terminally similar tufts of small leaves (Plate II, fig. 2). Accompanying this rosette form of injury is more or less yellowing, usually distributed over each of the young leaves, but occasionally confined on each leaf to irregular areas, separated by normal green tissue, producing a mosaic-like pattern.

The abnormalities which have brought about this divergence from the usual form of the plant may be analysed into the following symptoms: a cessation of the growth of the axis, a reduction in the length of the petiole and in the size of the leaflets, and chlorosis, malformation, and curling of the leaflets. We have found no evidence of true proliferation; although axillary buds appear to be forced into growth, and these, developing into short shoots with small crowded leaves, add to the general rosette appearance.

In our studies of the development of the disease, the first sign of rosette to be observed was a faint indefinite mottling of the youngest leaflets. The next leaf to open was predominantly of a pale yellow colour upon which the veins formed a green network. We found this distinctive character to be of certain diagnostic value for rosette at this stage (see the experimentally infected plant in Plate III, fig. 3). Later

formed leaves bore progressively smaller leaflets, chlorotic (often uniformly yellow, without dark veins), curled and distorted (Plate III, fig. 4). Elongation of the axis ceased after the appearance of the first chlorotic symptoms (Plate IV, figs. 5, 6).

Rarely we have found in the field single plants which exhibit both healthy and rosetted branches. Two plants in our experiments developed rosette in two basal shoots, while the main axis remained healthy. One of these plants, which was retained under observation, was still producing healthy leaves on the main axis 60 days after the first appearance of the disease. Normally the rosetted condition was present in every branch of the affected plant.

In the diseased plant, the yellow leaves, a few days after unfolding, started to become green and eventually reached a condition only slightly paler than a healthy leaf. This change occurred at all temperatures encountered. The transitory nature of the chlorotic phase affords an explanation of the irregularity of the observation of chlorosis in rosetted peanut fields. Generally it is a most noticeable character, splashes of pale lemon yellow showing up from a distance among the dark green foliage; although on other occasions a badly rosetted field may exhibit no marked yellowing. We believe that the latter condition is found when a check upon the growth of the plants has occurred, and that chlorosis is only in evidence when conditions are favourable to the rapid production of new leaves. In consequence of this "secondary greening" we sometimes encountered great difficulty with out-of-season plants in distinguishing rosette disease from the seasonal stunting to which the peanut plant is subject.

The occurrence of mosaic-like markings has shown considerable variability, and they are absent from what we regard as a typical rosetted plant. But in some plants the leaflets may exhibit a characteristic and pronounced mosaic pattern (Plate V, fig. 8 and Plate VI, fig. 9); this condition is usually accompanied by less severe stunting symptoms. We have kept in mind the possibility that we may have been dealing with two separate diseases; but for reasons which we discuss later we regard the two types as different manifestations of infection by the same virus. Mosaic-like markings were noted in East Africa by Zimmermann (10).

We have observed no case of true recovery from the disease, although, as already noted, at certain seasons rosetted plants may be scarcely distinguished from those stunted by other causes.

The rosetted plant may flower, but few of the pegs make any growth

and none bear seed. The only yield from diseased plants is the seed which had formed before the plants became diseased; where infection has occurred early in the plant's growth, the crop is a total loss.

Transmission.

Direct methods.

By the seed. The experience of farmers opposes the idea that rosette may be carried in the seed. Cases have been reported where the self-sown seed of a crop, so badly diseased as not to justify reaping, has in the following season produced a healthy stand. The very severe rosette season of 1922–23 was followed by a year of comparative freedom from the disease, although local seed was largely planted.

Our rather limited experimental evidence tends to confirm this conclusion. During the 1923–24 season, 330 seedlings were raised from seed of selected rosetted plants which was sown at monthly intervals from September to February in the open at Pretoria, and 40 in the greenhouse; all seedlings remained free from rosette. In 1926, 90 seeds from diseased plants were sown in a gauze-protected greenhouse at Durban and produced 86 healthy plants, only four seeds failing to germinate. There was therefore no evidence to suggest that possible virus-bearing seeds might undergo delayed germination and so escape notice.

It should be noted, however, that infection by the rosette virus inhibits further seed production by the plant, and that the seed used in the foregoing experiments was actually that formed during the healthy period preceding infection. Although, therefore, from the practical point of view, transmission by the seed appears to be excluded, the theoretical possibility of an invasion of the ovule by the rosette virus is not disproved.

By the soil. The field evidence, in this case also, opposed the idea that the rosette virus might survive to any considerable extent in the soil. In the 1923–24 experiments, 65 peanut seedlings were raised in boxes containing soil which had borne rosetted plants and 12 in boxes containing a mixture of soil and the remains of dead rosetted plants. No rosette developed in any seedling.

By juice inoculation. Rosette disease has proved not to be readily transferred by the direct inoculation of juice. Forty-five plants inoculated by the injection of the juice of diseased plants into the region of the growing point failed to develop rosette. Similar results were ob-

tained with 24 plants inoculated through wounds on the leaves and with a like number inoculated by insertion of small portions of diseased tissue into the stem.

By grafting. In a single experiment we have demonstrated the transmission of rosette by grafting. In August 1926, small shoots from diseased plants were grafted into three healthy peanut plants kept within an insect-proof greenhouse; rosette made its appearance in all three plants within 26–60 days from the date of grafting. Three control plants, grafted with apparently healthy scions, remained free from rosette symptoms during the same period.

Insect-vectors.

In the regions of severe outbreaks of rosette the peanut crop is subject to frequent heavy infestations of *Aphis leguminosae* Theo.[1] Although these infestations were often not followed by outbreaks of rosette, there was, at the time when we began these investigations, a tendency for farmers to associate rosette with aphid-attack. Preliminary trials in the open in 1924 supported this suspicion, and the experiments described hereunder demonstrate that this species of aphid is a transmitting agent of the disease.

No other species of aphid was found by us on peanuts in this region. Two species of leafhoppers were abundant in 1924–25 in peanut fields, but failed to transmit rosette in our experiments.

Methods. In the transmission experiments here reported, the insects were manipulated in glass tubes according to the method already described by one of us (9). The introduction of the insects into their tubes took place in the laboratory away from the greenhouse. A single mature leaf of each experimental plant passed through the wool plug which closed one end of the tube, and upon this leaf alone were the insects allowed to feed (Plate V, fig. 7). At the conclusion of the period of exposure the petiole was severed below the wool plug. The plants were in consequence protected from any general direct injury resulting from aphid or other infestation, and the disease was diagnosed from symptoms appearing in the parts of the plant upon which no insects had fed. This technique, devised originally for the manipulation of leaf hoppers, proved equally effective in aphid-studies.

The experimental plants were raised from seed and retained throughout the experiments within greenhouses with gauze-protected windows. The preliminary Pretoria experiments suffered from some white fly and

[1] Determined by Dr J. T. Potgieter, Division of Entomology.

red spider infestation, but later experiments were carried out under conditions of apparent freedom from extraneous insect infestation, except that red spider in certain cases became established upon the older plants. When this occurred, the house was emptied of all plants as soon as the current experiments were finished, and was thoroughly cleaned. In addition, a few small colonies of *Aphis leguminosae*, believed to have originated in very young forms which escaped during the manipulation of the tubes, became established; these were dealt with by destroying the plants infested and discarding the experiments concerned. Under the conditions of frequent close observation it was improbable that any insects, particularly aphides, might remain long unnoticed; and, that any insects present might have full opportunity to make themselves evident, no fumigation of the houses was practised. Control plants, from the same sowing and growing in the same soil containers, were retained under observation alongside the experimental plants. No control plant developed rosette.

The peanut variety, Virginia Bunch, was used throughout this work. Owing to the necessity for shading to prevent too high temperatures

Table I.

Tests of leafhoppers for rosette transmission[1].

Hoppers collected in rosetted peanut fields, Waterberg district. Tested in groups of six individuals or less on peanut seedlings.

Name of leafhopper	Total No. of hoppers tested	No. of seedlings upon which tested	No. of seedlings infected
CICADELLIDAE:			
Agallia nigrasterna Cogan	73	24	Nil
Empoasca sp. (*a*)	89	16	,,
Eutettix sp. (*a*)	3	3	,,
Erythroneura sp. (*g*)	5	3	,,
,, ,, (*h*)	1	1	,,
,, ,, (*k*)	1	1	,,
Euscelis obscurinervis Stal.	1	1	,,
,, sp. (*a*)	1	1	,,
,, ,, (*b*)	1	1	,,
,, ,, (*c*)	1	1	,,
Eugnathodus auranticulus Naude MSS.	1	1	,,
Thamnotettix sp. (*a*)	1	1	,,
FULGORIDAE:			
Dicranotropis maidis Ashm.	3	3	,,

[1] Determination made by Dr T. J. Naude, Division of Entomology. The lettering of the undetermined species refers to type specimens in his collection.

and to protect the glass tubes from direct sunlight, the plants were somewhat drawn, but they were of a good colour and not unhealthy.

By leafhoppers. During the season 1924–5, collections of leafhoppers were made by sweeping in fields of diseased peanuts in the Waterberg district. The insects were taken to Pretoria, and all that survived were tested immediately for rosette transmission (Table I). Two species only —*Agallia nigrasterna* Cogan and *Empoasca* sp. (*a*)—were caught in sufficient numbers to allow of a critical number of tests. It is likely that several of the other species were chance stragglers in the peanut fields, and of many only single individuals could be tested.

Although no rosette infection resulted from any of these trials, and the indication of the work was to exclude leafhoppers as vectors of rosette, yet this negative evidence is clearly not conclusive. Many of the insects died within a few days of the beginning of the experiments, and it is not certain that all actually fed upon the exposed leaves of the experimental plants. Furthermore it is not impossible that some of the insects might be carriers of the virus but unable to inoculate it into a mature leaf, to which alone, by the method of experiment, they were allowed access. This is however not probable, for the plant is susceptible to inoculation through an old leaf, as the aphid experiments show.

By aphides collected upon diseased plants in the field. Colonies of *Aphis leguminosae* Theo. were collected during the seasons 1924–5 and 1925–6 upon rosetted plants in the Waterberg and Natal coastal areas. Groups of aphides were tested upon peanut seedlings, and in all experiments produced rosette disease in a proportion of the seedlings, as shown in Table II.

Under the conditions of the experiment the plants did not develop the compact rosette form typical of this disease in the field. We consider this to be mainly due to the somewhat drawn condition of the experimental plants, already mentioned. But the plants believed to be infected showed plainly the symptoms which we have accepted as characteristic of rosette disease; the peculiar dark-veined chlorotic leaves (Plate III, fig. 3), the cessation of axial growth (Plate IV, figs. 5 and 6) and at a late stage the uniform chlorosis, dwarfing and curling of the young leaflets (Plate III, fig. 4). These experimental plants were as effective as naturally rosetted plants in infecting aphides which fed upon them (see the following section).

By aphides experimentally infected with the rosette virus. Early in these investigations we established a culture of *Aphis leguminosae* Theo. which was uninfective. This culture was maintained by transfers as

Table II.

Transmission by Aphis leguminosae *Theo.*

Aphides collected in the field upon rosetted peanut plants. Several aphides, in different stages of development, tubed on single leaves of peanut seedlings for 7–14 days. First symptoms observed after 12–40 days; plants retained under observation 60–125 days. Control plants growing alongside throughout period of observation.

Date	Locality where aphides collected	No. of aphides on each plant	No. of plants tested	No. of plants infected
Season 1924–25:				
25. i. 25–7. iv. 25	Waterberg District, Transvaal	About 12	71	32
	Controls	—	71	Nil
8. iv. 25	Natal Coast	About 10	6	5
	Controls	—	6	Nil
Season 1925–26:				
15. ii. 26	Waterberg District, Transvaal	About 5	14	2
	Controls	—	23	Nil
4. iii. 26	Natal Coast	About 6	24	22
	Controls	—	14	Nil

Table III.

Infection of Aphis leguminosae *Theo. with the rosette virus.*

Aphides, all from a single culture reared on healthy peanut plants, were fed on rosette-diseased plants, either tubed upon single chlorotic leaves or caged upon the whole plant, and tested in groups of six for about 7 days upon healthy peanut seedlings. Rosette appeared in 20–40 days. Control plants grew alongside.

Date	Nature of infection plant	Days on infection plant	No. of plants on which groups of aphides tested	No. of plants infected
9. iii. 26	Rosetted transplant from Waterberg (cage method)	4	12	4
	Controls. No aphides	—	13	Nil
9. iv. 26	Chlorotic leaves of rosetted transplant from Natal Coast (tube method)	11	6	4
	Controls. No aphides	—	7	Nil
10. vi. 26	Experimentally infected plant (cage method)	About 10	10	6
	Controls. No aphides	—	10	Nil
28. ix. 26	Plant experimentally infected on 15. ii. 26. Producing new chlorotic leaves after over-wintering (cage method)	21	12	4
	Controls. No aphides	—	15	Nil
Control tests of aphides:				
9. iii. 26	Aphides taken directly from culture cages	—	6	Nil
27. x. 26	ditto	—	12	Nil

necessary of a few aphides to caged healthy peanut plants. No rosette ever appeared in any of these food-plants; and tests of these aphides were made, in experiments in March and October 1926, in which they failed to infect any experimental peanut plants (see Table III, Control tests of aphides).

Aphides from this culture were allowed to feed for varying periods upon rosette-diseased peanut plants, including diseased plants transplanted from the field and others infected during earlier experiments in the greenhouse. In certain cases the aphides were confined in their feeding to single chlorotic leaves; otherwise they were caged upon the whole plant. The tests of these aphides (Table III) show that this species may acquire the virus of rosette disease by feeding upon a diseased plant.

By individual aphides feeding singly. Early experiments, in which single aphides taken from cultures were tested for transmission, gave only a single infection in 18 tests. A higher proportion of infections was obtained however with aphides collected upon diseased plants in the field. In an experiment of January 6th, 1927, adult aphides from a rosetted plant in the Natal coastal area were tested singly upon peanut seedlings. Of 15 apterous individuals tested, 3 caused infections, while 21 winged individuals caused 3 infections.

THE SIGNIFICANCE OF THE MOSAIC-TYPE OF THE DISEASE.

It has already been noted that in diseased peanut plants in the field the chlorosis of the young foliage may embrace the whole of every leaflet, or may be interrupted by areas of normal green tissue; and that in the latter case the mottling is generally accompanied by less severe stunting and a correspondingly less pronounced rosette character. These features are well shown by the two plants, of the same age and infected at about the same time, photographed in Plate V, fig. 8. In some cases the chlorotic part has hardly exceeded a tenth of the total leaf area.

This disparity raised doubt as to whether we were studying two separate diseases. Our field observations have led us however to believe that the two types are the result of the different reactions of individual plants to a single virus, rather than the result of two separate viruses. Although the extremes present a very striking contrast, we have found in the field many intermediate forms. In lightly infected plots, we have usually found the diseased plants in groups, which suggested a single source of infection for each group. Nevertheless, in a single group both types of disease have frequently been in evidence.

In our aphid-transmission experiments we have never obtained the extreme mosaic-type of the disease. But we have frequently noted a tendency towards this type (note, for example, the isolated green areas in the otherwise chlorotic leaf of the plant illustrated in Plate III, figs. 3 and 4). Such mottled plants have been obtained from transfer from fully chlorotic plants; while aphides taken from pronounced mosaic plants in the field have produced an unmottled condition in experimental plants.

Our conclusions have received support from observations of a mosaic-type plant which appeared in a plot at Durban. Large quantities of infective aphides were scattered upon the young seedlings in this plot, almost all of which within a month became diseased. The mosaic character was showing in one plant at this time; and, although subjected to repeated reinfection by virus-bearing aphides, this plant continued to produce mottled leaves. The photograph in Plate V, fig. 8, is of this plant at four months old, alongside a normally rosetted plant of the same age.

An examination of the leaves of diseased plants of the two types shows a difference only of degree; that whereas in normal rosette the chlorosis embraces the whole of the leaflet, in the mosaic form a similar chlorotic condition is confined to portions of the leaflet. We judge therefore that the mosaic form occurs in plants which are able to react against the virus in such a manner as to cause its effects to be localised and not general in the leaflets.

Studies of the disease under field conditions.

Observations in the Waterberg area of the Transvaal caused us to distinguish two phases of rosette infection: a localised phase, where the disease was confined to the plants within a limited number of clearly marked areas in the field, and an epidemic phase, in which the disease was generally distributed through the field. The localised phase we have come to associate with early sowing, the epidemic phase with late sowing. Where the disease has been observed in fields which were sown in November, it has usually been confined to a small number of groups of plants. These diseased groups are of striking appearance and may frequently be individually of considerable extent (Plate VI, fig. 10); within the limits of the affected area almost all the plants are diseased and severely stunted, while the margins, if the crop be nearing maturity, show a clear-cut line dividing the stunted plants from those which are healthy or have contracted rosette only after making considerable

growth. Farmers have reported cases to us where they were able to watch in the early stages a gradual advance of the disease at the margins of the areas. Usually in the early sowings the disease remains confined to the localised groups until the maturity of the crop. Late sowings are usually subject to epidemic infection.

In that we find no uniformity of distribution of the diseased areas from season to season nor any obvious relation to the soil conditions, we deduce that the areas are not the result of some predisposing soil factor; and on similar evidence and our experimental results we exclude the possibility of a localised soil infection. We judge them to have arisen by a spread of infection from a centre.

The centre of infection cannot be generally a diseased plant which has overwintered (in the manner to be described), for the diseased groups are commonly seen on land newly broken and under rotation; nor, in view of our experimental evidence, is it probable that the centre can be an occasional seedling which arises diseased from an infected seed. We advance the hypothesis that the diseased group is originated by a winged infective aphid, which, settling upon a young seedling of the crop, infects it with rosette; and that later the disease is carried to adjacent plants by aphides which have crawled from colonies established upon this central diseased plant. The epidemic phase is a consequence of a later general dissemination of winged aphides from colonies upon rosetted plants of the early crop. This hypothesis is in conformity with what is known of the life history of species of the genus *Aphis*; that the winged forms, which reach plants in spring, produce colonies which are mainly wingless, and that this process continues through several generations until at a later stage there occurs a new dissemination of winged forms.

The discovery of the source whence the original aphides in the spring obtained the virus of the rosette might well have an important bearing upon the problem of control. Our field studies, although rendered incomplete by the distance from our headquarters of the affected region, have led to the hypothesis that the virus survives in over-wintering peanut plants in the fields, whence the aphides become infected in the spring.

The peanut plant behaves as a true annual, and dies after ripening its seed. There is therefore no possibility of the survival of a perennating root-stock from the previous season's crop. But young plants which enter the winter before they have fruited may survive to the spring, although severely stunted and often damaged by frost. It is a common

experience that in late autumn much of the seed missed in harvesting will germinate; cases have been reported to us where fields in April have been green with this volunteer crop. We have ourselves found in July and September in the Waterberg area stunted but living survivors of this autumn germination.

The autumn volunteer plants appear at a time when the intensity of rosette infection is known to be great. We have reports of such plants growing amidst aphid-infested drying haulms of a ploughed-in diseased crop; of recognisable rosette in volunteers in April, in August (when they were making new growth after rain), and in September and later months. Ordinarily during the winter these plants appear to be making no growth, and, owing to the "secondary greening" effect (p. 29), rosette is not certainly recognisable in them. Experimentally the winter survival of rosette-infected plants was demonstrated in Durban. Seed sown in March 1926 produced plants which were infected by the distribution of virus-bearing aphides and showed rosette symptoms. During the winter these plants made no growth and rosette was not recognisable, but new foliage in August was typically rosetted (Plate VI, fig. 11). In a similar experiment at Potgietersrust, Transvaal[1], a plot, sown in February 1926, showed rosette within a short time; these plants were observed by us in July to be living, though frosted to the ground; but they were reported to have died in August after making some new growth. In the greenhouse at Durban, a plant infected on February 15th, 1926, survived the winter without making new growth until August. Aphides fed upon it in September became infective (experiment of September 28th, 1926 in Table III).

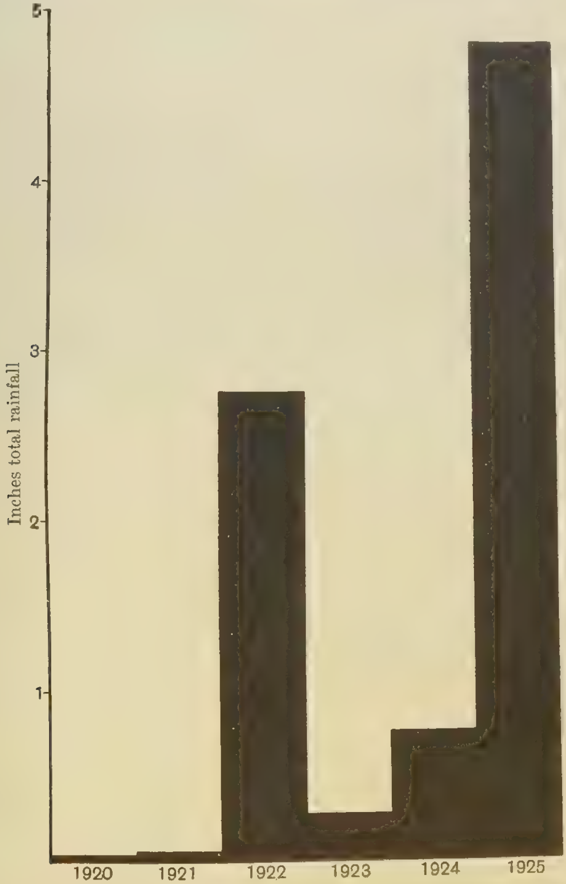
Although our only complete evidence of the overwintering of rosetted plants is confined to the comparatively mild conditions of Durban, yet there is strong indication that this overwintering occurs also in the Transvaal. It may be noted that the importance of out-of-season plants in carrying-over virus diseases has been recognised in other cases: for example, the wintering of the curly-leaf virus in unharvested beets (Carsner and Stahl (3)), and the summering of spinach-blight in surviving spinach plants (McClintock and Smith (6)).

We have failed to discover any alternate perennating host-plant of the rosette virus, and if such occurs it is either rare or carries the virus without exhibiting visible signs. The occurrence of rosette in peanuts planted upon isolated newly-broken fields, does not necessarily imply that infection has passed from some wild plant, since winged aphides may

[1] On the farm of Mr A. Gilbertson, to whom our thanks are due.

well be carried by wind from old peanut lands, even at a considerable distance.

We have not at any time discovered this aphis upon any wild plant



Text-fig. 1. Total winter rainfall (June to September) for the years 1920–25. Mosdene, Naboomspruit.

of the peanut region, but with difficulty we were able to find small colonies upon overwintering peanut seedlings in the months of July and September in the Waterberg. It is likely therefore that the winter is passed in slow agamic reproduction upon the autumn-germinated peanut

plants. In some cases the aphides may overwinter actually upon diseased plants, in other cases the diseased volunteers may become colonised in the spring; in either case eventually a dissemination will occur of winged aphides, frequently infective, as our experiments have shown, when feeding singly. We judge that the intensity of the spring infection will be determined by the numbers of infective aphides which thus reach the peanut fields.

If we have arrived at a correct explanation of the manner of overwintering of the rosette virus, then any circumstance favouring the winter survival of self-sown peanut seedlings may be expected to cause an intensification of the rosette infection in the following season. Rains during the normally dry winter would favour the survival of these plants.

We have been supplied by Mr E. E. Galpin with meteorological and crop data for the farm Mosdene, Naboomspruit, for the period 1920–26, during which peanuts have been grown on a commercial scale in this region. In respect of the severity of rosette in the November-sown plantings on this farm, the seasons fall into the following classes:

1922–23 and 1925–26. Severe rosette.

1924–25. Slight rosette.

1920–21, 1921–22 and 1923–24. Very little rosette.

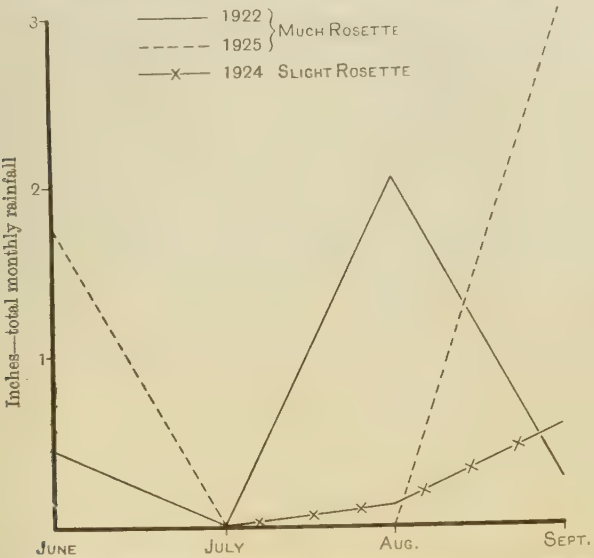
We have accepted, as the normally dry winter period, the four months, June to September, rain having fallen in all years in October, and in all but one in May. In Text-fig. 1 we show the total winter rainfall for these years. It is evident that the seasons of severe rosette followed the winters of exceptionally heavy rainfall, while dry winters were followed by seasons nearly free from rosette in the early sowings. The data for the season 1924–25 are possibly significant as showing an intermediate value.

The meteorological and crop data for a farm in the Potgietersrust area (about 30 miles from Mosdene) exhibit the same features as those already considered.

The rainfall of the three wettest winters shows a great irregularity of distribution (Text-fig. 2) and it is not possible at this stage to judge the influence of the separate monthly contributions to the total. The year 1925 shows however that a two-months drought (July and August) is insufficient to check the overwintering of rosette.

These data in our opinion afford a strong indication that the severity of the early rosette infection is determined by the preceding winter rainfall. On the other hand, we have failed to detect any relation

between rosette outbreaks and the weather conditions prevailing during the growing season. That these immediate weather conditions, with their known great influence upon aphid-development, must affect rosette spread is obvious. But we argue that if many diseased plants survive to the spring, then in any season there will sooner or later occur conditions which are favourable to aphid-colonisation of these plants and



Text-fig. 2. Monthly rainfall for three winters preceding seasons in which rosette appeared in spring-sown peanut crops. Mosdene, Naboomspruit.

to the dissemination of winged aphides from them. Nor have we found evidence in the data available to us in support of the view, widely held by farmers, that any check on the plants' growth, whether by excessive rain or drought, encourages rosette. Rather we would judge that a sudden striking manifestation of any rosette disease already in the plants is produced in the renewal of growth after a check.

Control.

The experience of farmers has clearly demonstrated that there is small prospect of any return from peanuts sown late in the season in the regions subject to rosette disease. The greatest hope of escaping severe infection is to be found in planting as soon in each season as the

soil moisture and temperature will allow of good growth. In the Waterberg this best sowing date is generally about the middle of November.

In view of the findings in the preceding section it appears to be likely that the number of new infections in the early crops will be reduced by any measures taken to secure the destruction of volunteer peanut plants during the winter and in the spring before any plantings of peanuts are made. Since dissemination of aphides from a single group of rosetted peanut plants may occur in all probability over a wide area, the full effects of this policy are likely to be felt only if it be adopted by all farmers in a district. The individual however is likely to benefit to a considerable extent by thorough winter ploughing of his own fields and the destruction of volunteer peanut plants in headlands and elsewhere.

If our explanation of the manner of production of the diseased patches during the early stages of rosette infection be the correct one, then it is possible that spread may be checked by a system of roguing. Success would appear to depend upon the early discovery of the primary infections in the field, upon the destruction of these plants in such a manner as to avoid scattering the aphides developing upon them, and upon the simultaneous removal of contact plants. This procedure has not as yet been put to any considerable test, but promising results have followed certain preliminary trials.

There appears to be little prospect of any practicable form of general attack upon the aphides being successful in checking rosette outbreaks.

Whatever success attend direct measures for rosette control, it seems to be highly improbable that the peanut growing industry of South Africa can be placed upon a sound footing until a rosette-resistant variety come into cultivation. Unfortunately no such variety is available, nor have the early trials of a number of commercial varieties indicated any which are markedly more resistant to rosette than the standard Virginia Bunch variety.

The apparent correlation between the winter rainfall and the severity of spring rosette infection affords the farmer some indication of the season's prospects for peanut growing before the season opens. At present farmers in the Waterberg have lost confidence in this crop, and the uncertainty of realising any yield has told against economic marketing when a good yield has been obtained. Whether conclusions based on six years' results alone will be borne out by future experience is uncertain; but the farmer is now not entirely without a guiding principle in deciding upon the area which he shall put under peanuts. If the

rainfall for the four months, June, July, August and September, has been less than a quarter of an inch there appears to be a good prospect of escaping severe rosette damage to the November-sown crop. After a rainfall for the same period exceeding two inches the prospects are unfavourable.

SUMMARY.

Rosette is a destructive disease of peanuts in South Africa, characterised by chlorosis of the young leaves and extreme stunting. Similar conditions in this plant have been reported from tropical Africa, Java and India.

Experimental evidence is advanced to show that rosette is not carried in the seed of the peanut nor in soil which has borne a diseased crop. It was successfully transmitted to healthy plants by grafting, but not by inoculation of juice from diseased plants.

Insect transmission studies demonstrated the ability of *Aphis leguminosae* Theo. to act as a vector of rosette. Thirteen species of leafhoppers in a limited series of tests failed to transmit the disease.

Aphis leguminosae was shown to obtain the virus of rosette by feeding upon a diseased plant. Winged and wingless adults, when feeding singly, were shown occasionally to transmit the disease.

An infrequently occurring mosaic-like form of the disease is considered to be due to an exceptional reaction of individual plants to infection by the same virus as that causing typical rosette.

In the field this disease is believed to overwinter in diseased plants, which germinate in the late autumn and survive the drought and frost of winter. Upon these plants the aphides may spend the winter. It is thought that in the spring winged aphides become disseminated from the overwintering plants and cause localised infections in the peanut fields. Later in the season epidemic spread of rosette frequently occurs. The evidence of six years of peanut-growing indicates that spring infection of rosette is most severe in seasons following winters of exceptional rains.

Rosette disease is likely to be largely escaped in the average season if the crop be planted at the earliest favourable date. Precautions recommended for control are the destruction of surviving plants during the winter and the removal of the diseased plants which develop in the new crop. The best prospect of practical control of rosette however is thought to lie in the discovery of resistant varieties.

EXPLANATION OF PLATES II—VI.

Rosette disease in peanuts, variety Virginia Bunch.

PLATE II.

Fig. 1. The disease in the field. Healthy and diseased peanut plants, the latter infected at an early stage of growth.

Fig. 2. The disease in the field. Peanut plant infected at a late stage, showing rosettes borne on the ends of the branches. The spotting of the older leaves is due to other causes.

PLATE III.

Fig. 3. The experimentally-produced disease. Early stage in the onset of the disease, the young leaf exhibiting chlorosis with dark veins. Inset—a healthy young leaf of similar age.

Fig. 4. The experimentally-produced disease. The same plant as Fig. 3, fifty days later. Young leaves small, curled, chlorotic, without dark veins. Photographed from above. The spotting of the old leaves is unconnected with rosette.

PLATE IV.

Fig. 5. The experimentally-produced disease. The same plant as Fig. 3, at a slightly later stage. Showing cessation of growth of the axis. Compare with Fig. 6.

Fig. 6. Control plant to that in Fig. 5. Healthy.

PLATE V.

Fig. 7. Method of experiment with *Aphis leguminosae* Theo. in a gauze-protected green-house. (See description in text.)

Fig. 8. The mosaic-type of the disease. Mosaic plant in Durban plot, alongside normal rosetted plant, of the same age and infected at about the same time.

PLATE VI.

Fig. 9. The mosaic-type of the disease. Separate leaves from plants showing the mosaic-type (*a*) and normal rosette-type (*b*).

Fig 10. Group of diseased plants in a field, otherwise predominantly healthy.

Fig. 11. The overwintering of the disease. Diseased plant from seed sown in March, 1926, at Durban, infected soon after germination, photographed in November, after having survived the winter. Healthy new-season plant alongside.

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Fig. 1.

Fig. 2.

STOREY & BOTTOMLEY.—The Rosette Disease of Peanuts (pp. 26–45).

Fig. 1.

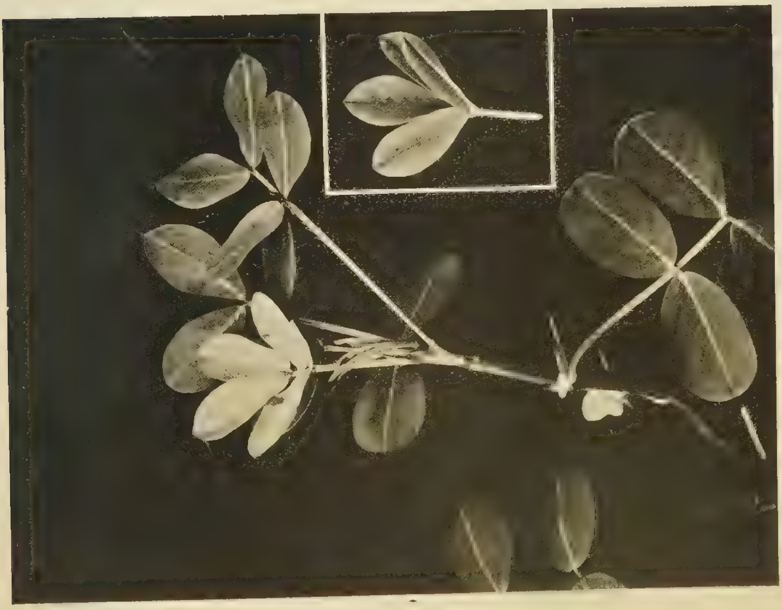

Fig. 3.

STOREY & BOTTOMLEY.—THE ROSETTE DISEASE OF PEANUTS (pp. 26 45).

Fig. 6.

Fig. 5.

STOREY & BOTTOMLEY.—THE ROSETTE DISEASE OF PEANUTS (pp. 26–45).

Fig. 7.

Fig. 8.

STOREY & BOTTOMLEY.—THE ROSETTE DISEASE OF PEANUTS (pp. 26–45).

b *b* *a* *a*

Fig. 9.

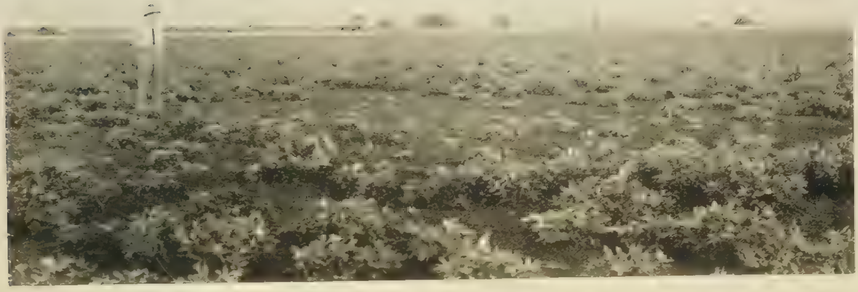

Fig. 10.

Fig. 11.

STOREY & BOTTOMLEY.—THE ROSETTE DISEASE OF PEANUTS (pp. 26–45).

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STUDIES IN BACTERIOSIS. XV

A DISEASE OF SWEDES AND TURNIPS

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(With Plates VII and VIII.)

CONTENTS.

(1) Introduction.

During the past two years diseased swedes on several occasions have been submitted to this department by the Ministry of Agriculture and others. Little is known as to the distribution or as to the extent of damage caused, but the fact that the disease has attracted attention in districts as widely separated as Hampshire, Cardiganshire and Ayrshire suggests a general distribution throughout these islands and considerable loss has been reported; in one case as much as 30 per cent. of the roots were badly decayed. The symptoms of the disease suggest to a certain extent the attack of *Pseudomonas campestris* (5) and, as the sequel will show, an organism closely resembling this has been isolated. At the same time there are certain features which make it a little doubtful whether the whole truth has been revealed and this paper is to be regarded therefore as a preliminary communication. The reasons for publishing it now are two-fold; firstly one of us (R. L. N.) is leaving the country and will be unable to continue the study of the disease when fresh material becomes available; and secondly, the organism isolated

corresponds exactly with one isolated in France from cauliflower by Dufrenoy and Szymanek (1) and, at the suggestion of Prof. Et. Foëx, submitted by those authors to this department for identification.

(2) THE SYMPTOMS OF THE DISEASE.

From reports received with the material it would seem that no sign of disease was discernible in the tops and no disease was suspected until lifting time. It was then seen that the underground parts of a large number of plants were in a very advanced state of decay. Quoting from a report of Mr D. W. Davies to the Ministry of Agriculture, "when these roots were cut across, the whole of the fleshy portion of many of them was decayed, but in others only the main tap root was affected, and from the brownish coloration of the tissue it seemed to be growing upwards towards the crown until the whole of the fleshy part was diseased. The growth of the rot, therefore, seems to indicate primary infection through the soil." When received in the laboratory the roots were mostly in a very advanced state of rot, as shown in Plate VII, fig. 1, where the flesh had become the prey of all manner of organisms and had been reduced to a slimy, pink, and evil-smelling mass. Roots in a less advanced state of decay showed blackened areas and hollowed-out cavities strongly suggestive of black-rot, but at the same time there were patches of brown tissue without the characteristic brown speckling of the vascular bundles which is associated with that disease. In what is believed to be the earliest stage of the disease, the tissue only appeared to differ from the normal in being stained pinkish-brown. Islands of this pinkish-brown tissue are seen in Plate VII, fig. 2, Plate VIII, figs. 3 and 4 at points marked *X*. It is the appearance of these streaks and patches, so unlike the accepted symptoms of early attack by *Ps. campestris*, that has led one, despite the fact that an organism similar to *Ps. campestris* has been isolated from them, to suspect that these streaks may have some other origin, possibly some physiological disturbance which has rendered the plant susceptible to the attack by the organism isolated. It will be seen, moreover, that the organism does not fit perfectly the description of *Ps. campestris* as given by Smith (5). Considering this and the absence of symptoms of black-rot in the leaves one cannot feel satisfied, without some further investigation, that the organism in question is correctly diagnosed as *Ps. campestris*.

(3) Isolation of the organism believed to be the cause of the disease, and inoculation experiments.

Some considerable difficulty was experienced in freeing from the accompanying saprophytes a pure strain with pathogenic properties. By selecting the drier patches of diseased tissue and inoculating these direct on to sterile slices of turnip, the disease could readily be transferred and in eight days would develop into a black rot of the tissue, without disintegration and without very marked selection of the vascular bundles. It was some weeks however before success attended the efforts to obtain a parasitic strain by plating from such diseased tissue. When eventually obtained, the pure culture inoculated on the surface of sterile turnip produced faint browning, reddish brown or sometimes deep brown on the first day at 25° C.; this would be blackish by the sixth day and intensely black on the eleventh day. The result of inoculation is shown in Plate VIII, fig. 5. The organism was found to cause a faint browning of cabbage stalk and the leaf veins became discoloured in eight days, but apart from this, all study of the effect on other cruciferous plants remains for future investigation.

Re-isolation of the organism was very satisfactory, agar plates showing by observation 60–100 per cent. colonies of the causal organism, which was successfully re-inoculated in about twenty instances.

In the course of these isolation experiments, there has frequently developed on the plates along with the parasite, a yellow organism with distinctly different properties. This organism called for more than usual attention since an exactly similar strain was found to be present as an impurity in the culture sent from France referred to above. This coincidence was striking enough, but specially interesting to the writers since at the time one of them (R. L. N.) was engaged on an investigation of saltation in bacteria, and it seemed possible that the yellow organism might be a variant of the parasite. Nirula's investigation (4) has, however, led him to the conclusion that no such relationship exists.

(4) Description of the causal organism.

A. *Morphological Characters.*

Form and arrangement. The organism in a hanging drop prepared from a young agar slant is a short rod of average length $1{\cdot}2\,\mu$ slightly rounded at the ends, and occurs very frequently in pairs and rather rarely in chains of 3–6.

Motility and flagella. The organism is fairly motile in both solid and liquid media and young and old cultures. An 18-hour old culture shows in a hanging drop very brisk motility of the sinuous swimming type, rarely straight, and frequently with

spells of quick and sudden tumbling on the short axis. Swimming goes on under a cover-glass for more than two hours and a half. A two-day old culture showed equal activity. An eight-day old culture on agar slant still showed fairly active swimming with sluggish tumblings. No difficulty was experienced in demonstrating the position and number of flagella. Gray's (2) method gave very satisfactory results. They were 1–4 at one pole (2 and 3 very common and 4 rather rare) (Plate VIII, fig. 6).

Behaviour towards stains. The organism stains fairly well with carbol fuchsine, ammonium oxalate gentian violet, victoria blue and methylene blue. It is gram negative and non-acid-fast.

Spores. These were not observed in any of the cultures; old cultures after being heated at 80° C. for 20 minutes did not subsequently show any growth.

Capsules. These were suspected, for the organism gets very sticky in old cultures, but they have not been demonstrated.

B. *Cultural Characters.*

In the following series of experiments, the temperature of incubation was 25° C.

Bouillon-agar plates (*p*H 6·8). The surface colonies showed in 36–48 hours as creamy-white glistening circular and raised (0·5 mm.); as seen with the lens they were spherical with a faint brownish tinge and showed striations which were not definitely concentric. On the third day there appeared a distinct central area with a yellowish tinge, which later on developed into a papillum. In a plate fairly thinly sown the colonies increased about 1 mm. each day. On the ninth day they were about 8 mm. in diameter. At this stage the colony was perfectly flat and circular with a central dirty white area surrounded by an inner pale yellow ring and an outer dirty white wet-shining area. The depth colonies were spindle-shaped and varied from 0·5 to 1 mm. in diameter.

Streak culture on bouillon-agar (*p*H 6·8). The growth was visible in about 30 hours in the form of crowded colonies (0·3 mm.) and on the whole presented a faint yellowish tinge. On the third day it had a central area, dirty white in colour and composed of fairly crowded colonies. Then followed on both sides a glistening zone of pale yellow made up of separate radiating folds and beyond this a dirty white zone perfectly smooth and with undulating margin. On the sixth day the central zone was yellow and was small in contrast to the outermost zone which was now 1 mm. on each side. On the ninth day the central zone and the next one were faint, while the outermost was prominent and distinctly wavy.

Stab culture in bouillon-agar (*p*H 6·8). In 24 hours there was a faint uniform growth on the surface; on the fourth day the surface growth consisted of conical colonies meeting at their pointed ends and the whole growth presented a yellowish tinge in the centre and dirty white outside. On the sixth day it became circular (1 cm. in diameter) and had a yellowish tinge all through. The growth in the depths of the stab was filiform on the first day, and developed into a ribbon-like growth with wavy margin.

Potato-mush plates. The shape of the colonies, the time of appearance and the structure were like those on bouillon-agar. Here, however, they developed on the third day a distinct pale yellow colour which was lost on the sixth day.

Potato-mush streak. The growth appeared on the second day, flat, moist and glistening about 3 mm. in width. It was distinctly chromogenic of pale lemon yellow

colour. The margin was slightly wavy. Later in the appearance of different zones it was like the one on bouillon-agar. On the fifth day the outermost area was 0·5 mm. on each side, flat, wavy and of dirty milky colour; then the layer with elongated transverse folds was rather indistinct and finally the innermost zone was of yellow colour and 2·5 mm. in width.

Bouillon-gelatine plates (*p*H 7·0). The colonies showed on the second day as whitish growth in cup-shaped depressions 0·5–1 mm. in diameter. On the third day they were rather thin with a thick outline and were placed in a very shallow circular depression (4 mm.). In a thin plate the colonies showed a central dirty white area surrounded by a thicker ring made up of small pieces projecting into the centre; outside was a transparent area of liquefaction. On the fourth day the liquefied areas ran into each other and were turbid with flat circular colonies floating in the liquid mass.

Stab culture in bouillon-gelatine (*p*H 7·0). On the third day there was a cup-shaped depression (0·5 mm.) on the surface with a whitish growth. In the medium it was filiform along the track of the puncture. On the fourth day there was a yellowish tinge in the growth at the bottom of the cup (2·5 mm.). On the fifth day it became cylindrical 3 mm. wide and 2 mm. deep. After four weeks the gelatine was not entirely liquefied, the solid portion occupied about 1·5 in. at the bottom. The liquid portion at the top 0·8 in. was slightly turbid with a thin whitish pellicle at the top and a mucoid precipitate at the bottom.

Streak culture on bouillon-gelatine (*p*H 7·0). Growth filiform of rather dry aggregated colonies on the third day. A distinct depression on the fourth day. Entirely liquefied on the tenth, showing thick sticky mass at the bottom of the fairly clear liquid above.

Glucose, sucrose and lactose agar shake cultures. The growth began on the third day as a faint, whitish, thin layer and assumed on the fourth day in its central part a faint yellow tinge. On the fifth day it spread on the glass surface above. But there was neither any growth, nor any gas in the medium after three weeks.

Nutrient bouillon broth (*p*H 6·8). The growth was fairly vigorous. Strong clouding of the medium took place in 24–30 hours. On the fourth day there was just the appearance of a faint ring easily washed off. On the tenth day it was continuous, of a pale yellowish white colour. On the twentieth day it had a central yellowish line enclosed on both the upper and lower sides by thin whitish layers. The pellicle began as a thick layer on the third day. It was not of uniform consistency and broke up into small sized particles which floated very freely in the medium. On the twenty-fifth day it was fixed firmly at the top as a thick dirty white mass.

C. *Physiological Characters.*

In the following inoculations a two-day-old culture was used and the tubes were incubated at 30° C.

1 *per cent. glucose and peptone broth* (with litmus). On the second day it was fairly turbid, showed a faint change in colour on the bluish side (alkaline) and had developed a thick membranous pellicle of whitish colour which sank to the bottom on the slightest agitation. On the fourth day the litmus was bleached in the lower half. On the eighth day only a shallow bluish layer remained at the top. On the twentieth day the medium was fairly clear with a greenish ring and a thick cottony pellicle. No gas.

1 *per cent. lactose and peptone broth* (litmus). Like glucose except that the pellicle was flocculent in its early stages and never became thick and cottony. The ring was bluish.

1 *per cent. sucrose and peptone broth* (litmus). Like glucose. Only here on the second day there were some flocculi in suspension caused through the breaking up of the pellicle and the litmus was not entirely bleached even at the end of a month. It had all the time a bluish layer (1 in.) at the top. The ring which began as a faint broken one with a whitish colour on the fourth day was bluish on the seventeenth. The pellicle was thick and cottony.

1 *per cent. glucose and peptone broth* (brom thymol blue). A faint ring on the first day washed down into the fairly turbid medium now distinctly alkaline (pH 6·8 changed to pH 7·1). On the fifth day the medium was deep blue. On the eighth day the pellicle was flocculent. On the twenty-fifth day a continuous ring of peacock green colour. No gas.

1 *per cent. sucrose and peptone broth* (brom thymol blue). More alkaline than in glucose on the first day. On the fourth day the pellicle was in one case flocculent and in another case was a shallow cup of bluish green colour with marked radiating lines in it. Otherwise like glucose.

1 *per cent. lactose and peptone broth* (brom thymol blue). Like sucrose, only the pellicle was of thick membranous structure on the second day. On the thirteenth day there was a faint whitish ring. On the seventeenth day there was not much of the pellicle and the ring was not so conspicuously developed as in sucrose, though it was quite distinct with a bluish colour. On the twenty-fourth day it had a greenish tinge.

Synthetic medium as suggested by the Society of American Bacteriologists in their Manual (brom thymol blue). The growth was very poor with all the three carbohydrates (glucose, sucrose and lactose). A very slight milkiness on the fifth day and a very faint bluish tinge on the eighth day. Nothing went further. No gas.

1 *per cent. maltose and peptone broth* (litmus). On the second day there appeared a thick and membranous pellicle in a fairly turbid medium now slightly bluish. On the fourth day there was a bluish white ring and white frothing scum. On the fifth day the litmus was bleached in the lower three-quarters of the solution. On the eighth day the pellicle had broken up into a number of long filaments hanging down into the medium and the litmus was entirely bleached. On the thirteenth day there was a thick cotton-like pellicle of dirty white colour with a faint yellowish tinge and a greenish ring which became slightly bluish on the twentieth day.

1 *per cent. mannite and peptone broth* (litmus). The medium on the second day was highly turbid, slightly alkaline and showed a broken pellicle. On the fourth day there was a faint ring in the now distinctly alkaline medium. On the eighth day the litmus was partly bleached and there was a perfectly continuous ring which had a bluish and greenish tinge, was white on the sixteenth day, became bluish with a dirty green tinge on the twentieth and was of a sky blue colour on the thirtieth day. The litmus was not entirely bleached even at the end of a month when an inch layer at the top was still bluish. The pellicle never became so thick as in maltose.

1 *per cent. peptone broth.*

(*a*) Fairly turbid on the first day. Further on practically like mannite. At the end of a month there was a discontinuous white ring, thick pellicle with small thread-like filaments hanging into the fairly turbid medium.

(*b*) No indole.

Diastatic reaction. Potato-starch plates showed dirty white growth on the first day which became pale yellow and glistening on the second day. Tested with iodine on the sixth day, the inoculated plate stained pinkish violet with pink areas surrounding each colony in contrast to the blue colour assumed by the control.

Potato plugs showed growth after 24 hours as moist and glistening with a distinct yellow colour. On the third day it became dirty yellow. On the sixth day it deepened to the "Saccardo's umber" in the one tube and "bister" in the other (colour descriptions are from Ridgway's Colour Standards). On the tenth day the pointed end became blackish. After a month it was brownish-black all over, was glistening and reduced in volume. 1 c.c. of the plug was taken, ground up in 100 c.c. of distilled water and tested with a few drops of iodine in test tubes. On the sixth day, seventeenth day, and at the end of one month and a half, the inoculated one showed a purple colour in contrast with the distinctly blue colour in the controls. These tests showed the organism to be possessed of decided though moderate diastatic action.

Uschinsky solution (brom cresol purple and cresol red). The growth was very poor. The medium assumed slight turbidity on the fourth day, showed a faint acidity on the tenth day and was distinctly acid on the twenty-seventh. At the end of one month and a half the solution was fairly clear without any pellicle, ring, or noticeable precipitate.

1 *per cent. nitrate broth.* Fairly turbid in 24 hours. Highly so on the third day. Nitrate reduced distinctly in 24 hours. At the end of one month only small traces of nitrite were present owing to the formation of NH_4 which made its appearance first on the fourth day. A pellicle appeared as a thin white ring-like growth on the third day and became very conspicuous, thick and membranous on the fifth day. The ring started at the same time, but was till the end of one month quite faint, though nearly continuous. The pellicle fell to the bottom on the slightest agitation and there was a large amount of it in 20 days.

Fermi's solution. The growth was rather poor, showing on the second day a faint milkiness in the medium which became fairly marked on the fourth day. At the end of one month it was quite clear and perfectly transparent as the control, but on shaking it became distinctly turbid.

Cohn's solution. As described for Fermi's solution.

Litmus milk. On the second day extremely faint change in colour, on the side of alkalinity. On the fifth day distinctly alkaline in the upper centimetre. On the sixth day the separation of the whey was visible to a depth of 1·2 cm. Alkaline layer at the top. On the eighth day the litmus was slightly bleached, the bluish tinge being present only at the top and the soft casein had fallen down; on the eleventh day there was at the top along the walls of the tube a ring (3 mm.) of "peacock green" colour. Then the whey was fairly clear to a depth of 0·8 in. Next came a mucoid layer of dirty mineral grey colour 0·5 in. in length. Lower down was the soft casein of fainter lavender colour with some cracks here and there. On the fifteenth day the colour of the litmus was regained and there was a mucoid layer and a chalky precipitate which became very conspicuous at the end of three weeks. No peptonisation even at the end of one month and ten days. The curd was very soft.

Milk. It showed changes just like the above in the coagulation of casein, the separation of whey, in the formation of the mucoid layer, the very soft curd which

never became a compact column, and the chalky precipitate which showed as speckling in the mucoid layer.

Milk with methylene blue. The deep blue colour of the milk changed to "porcelain blue" in 24 hours and the top inch layer was practically bleached. On the second day there was only a trace of colour at the top and at the bottom. On the sixth day the colour was slightly restored along a 3 cm. layer from above downwards. On the eighteenth day there was a blue colour with deep blue patches in it at the top, a soft curd below and a very dirty turbid whey. On shaking the colour became greenish blue all through.

Thermal relations. The thermal death point lies between 52°–53° C.

The maximum temperature for growth is 37° C. and the minimum is 8° C. The organism is fairly uniformly active in its growth at 20° C., 25° C. and 30° C.

Relation to moisture. The organism is not very sensitive to drying. Loop transfers were made from a 48-hour-old culture on sterile strips of mica kept at air temperature in the dark. One of these strips was transferred daily to a melted bouillon-agar tube and plated. For the first three days the plates became crowded with colonies but after this time the organisms were dried on to the mica in such a way that they were not easily removed; development then occurred only in the immediate neighbourhood of the mica strip. These air dry organisms continued viable and gave colonies beyond twenty-five days.

(5) IDENTITY OF THE ORGANISM WITH THAT OF DUFRENOY AND SZYMANEK AND ITS RELATIONSHIP TO *PSEUDOMONAS CAMPESTRIS*.

Before proceeding to discuss this question, a comparison of the present species and *Ps. campestris* is given.

Ps. sp.?	*Ps. campestris*
(1) Short rod with rounded ends	Short rod with rounded ends (Smith) (5)
(2) $1{\cdot}2\mu \times 0{\cdot}3$–$0{\cdot}6\mu$	$1{\cdot}1$–$2\mu \times 0{\cdot}5$–$0{\cdot}7\mu$ (Mehta) (3)
(3) Usually in pairs Also singly Occasionally in chains of 3–6	Singly (Mehta) Singly or in pairs (Smith)
(4) Actively motile by 1–4 polar flagella	Actively motile by 1 polar flagellum
(5) Gram negative	Gram negative
(6) No spores	No spores
(7) Markedly aerobic	Markedly aerobic
(8) Capsule (not demonstrated)	Not capsulated
(9) Faintly chromogenic (Pale yellow on agar: lemon yellow on potato plug)	Distinctly chromogenic (Pale to deep yellow pigment)
(10) Surface colonies on agar or gelatine coming very quickly	Rather slow growing
(11) Growth in stab cultures best near the surface	Growth in stab cultures best near the surface
(12) Gelatine liquefied very quickly	(*a*) Liquefied very slowly (Smith) (*b*) No liquefaction of unfavourable gelatine (Smith) (*c*) No liquefaction (Mehta)

Ps. sp.?	*Ps. Campestris.*
(13) White colonies on agar with a dirty yellow papillum and striations	Yellow colonies No papillum No striations
(14) Nitrate reduced to nitrite (very quickly)	Nitrate not reduced (Mehta) Nitrate not reduced (Paine and Nirula) Reduced (Smith)
(15) Diastatic action (sufficiently moderate)	Strong on the 15th day (Mehta)
(16) No indole	No indole (Mehta) Slow production of indole (Smith)
(17) Scanty growth in Fermi's solution. Uschinsky's solution and Cohn's solution	Scanty growth in all the three (Smith)
(18) Uschinsky's solution—acid and no gas	Alkaline and no gas (Mehta)
(19) No gas in peptone broth + mannite or maltose	No gas (Smith)
(20) Sugar media (glucose, sucrose, lactose) Alkaline (distinct and quick) No gas	 All three alkaline (Mehta) No gas
(21) Litmus milk Alkaline Coagulation + (mucoid layer and chalky precipitate) Digestion of casein?	(*a*) Alkaline No coagulation No digestion of casein (Mehta) (*b*) Casein precipitated slowly Gradual digestion of casein (Smith)
(22) Resistant to drying	Resistant to drying
(23) Thermal death point 52°–53° C.	48°–50° C. (Mehta) 51° C. (Smith)

The two organisms were compared most carefully in all their characteristics with each other and with the stock culture of *Ps. campestris.* In all respects the French culture differed from the stock culture in exactly the same way as did the organism from the Swede disease. Perhaps a slight difference was found in the number of flagella, organisms with more than one being fewer than in the case with the "Swede" organism. This difference seems insignificant since by stained preparations made by the present authors it was firmly established that in the French strain some individuals certainly existed with more than one flagellum (see Plate VIII, fig. 6 A).

The possession of more than one flagellum does not in the authors' opinion mark this organism as distinct from *Ps. campestris*. Smith (5) in his later writings expressed himself as uncertain on this point.

There is then no difference between our organism and that of Dufrenoy and Szymanek, and the question of their relationship to *Ps. campestris* will now be considered. The foregoing has shown how similar are the organisms and the points in which they differ from the stock culture. The chief differences are in the colour and contour of the colony on agar, the rate of liquefaction of gelatine, and the rate of growth on all media. These differences are so marked and obvious that

at first one was led to regard them as distinct species. The differences however are of the same order as certain differences found by Nirula (1) in saltants which arose in laboratory culture of bacteria. In the light of this work therefore one hesitates to assume that the organism is an entirely different species from *Ps. campestris*, one rather inclines to the view that some sort of saltation has occurred in nature and that the two are biological strains, either one having arisen from the other or both having sprung from some common parent.

(6) SUMMARY.

(1) A disease of swedes is shown to be related to the presence of bacteria.

(2) Certain features of the disease lead the authors to reserve their opinion as to whether bacteria are wholly responsible until opportunity for further investigation shall have presented itself.

(3) An organism has been isolated and described.

(4) The organism has been shown to be identical with one isolated contemporarily from cauliflower in France by Dufrenoy and Szymanek.

(5) The identity of the organism with *Pseudomonas campestris* has been discussed and it has been suggested that it is probably a saltant strain of this species, though the authors reserve the question of actual identity until further evidence is available.

(6) The strain possesses more than one flagellum.

EXPLANATION OF PLATES VII AND VIII

PLATE VII.

Fig. 1. The final condition of a diseased swede.

Fig. 2. Showing a typical case of a diseased swede with the blackened and hollowed area near the crown, and showing at points marked *X* pinkish brown areas believed to be the initial stage of the disease.

PLATE VIII.

Fig. 3. A typical case of diseased swede.

Fig. 4. A typical case of diseased swede.

Fig. 5. The result of a surface inoculation of a sterile slice of turnip with a pure culture of the suspected organism. (The negative was accidentally cracked.)

Fig. 6. A stained preparation of the suspected organism showing the presence of more than one flagellum.

Fig. 6A. A stained preparation of the organism of Dufrenoy and Szymanek believed to be identical with the organism suspected as the cause of this disease of swedes.

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Fig. 1.

Fig. 2.

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Fig. 3.

Fig. 4.

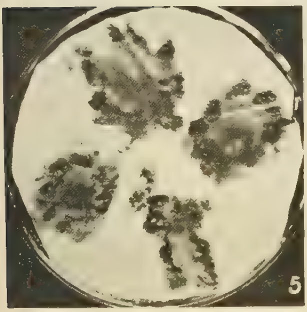

Fig. 5.

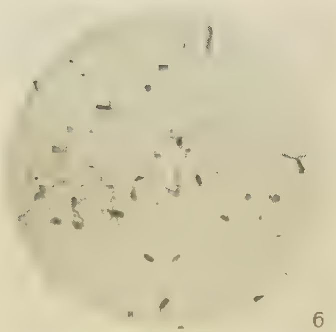

Fig. 6.

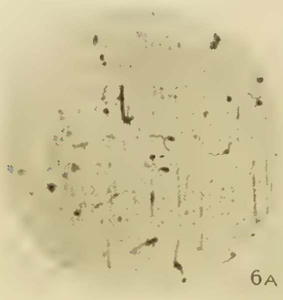

Fig. 6A.

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THE DECOMPOSITION OF NAPHTHALENE IN THE SOIL AND THE EFFECT UPON ITS INSECTICIDAL ACTION

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(With 4 Diagrams and 1 Text-figure.)

CONTENTS.

Introduction.

Naphthalene, either alone or in conjunction with other materials, has a certain reputation as a soil insecticide. It has been recommended for use against wireworms and leather-jackets; many experiments, however, have shown that its toxic action is uncertain under field conditions. Data to be presented indicate that in pot experiments in which thorough mixing of powdered naphthalene with finely divided soil is carried out by hand, naphthalene at moderate

concentrations is toxic to wireworms. Failure on a large scale may therefore be due to one or all of the following causes: (1) imperfect incorporation with the soil; (2) rapid disappearance from the soil either by decomposition or volatilisation; (3) a repellent action causing migration of the insects to positions where the vapour of the chemical is no longer effective. Naphthalene is known to be negatively chemotropic to many insects.

It has been shown (8) by experiments in sealed flasks that an atmosphere saturated with naphthalene vapour is not toxic to wireworms in a thousand minutes, its action being limited by the low vapour pressure of the compound. Thus naphthalene has three draw-backs for use in the soil as an insecticide: (*a*) it is slow in toxic action; (*b*) its vapour diffuses only slowly through the soil, in consequence of which its zone of toxic action is limited to a small volume; and (*c*) insects on coming into its zone of action are repelled and may escape from its toxic effects, unless the chemical is incorporated thoroughly with the soil to a fairly good depth.

It was early noticed in pot experiments that naphthalene disappeared from a good garden soil at a rate too rapid to be entirely accounted for by volatilisation. It appeared probable that decomposition by the action of micro-organisms in the soil was taking place. If this were so, the rate of loss would be expected to vary with the type of soil, and in certain soils the naphthalene might be decomposed too rapidly to be effective as an insecticide. Further, if the disappearance were due to bacterial action, the increasing numbers of naphthalene-decomposing organisms, which would result from repeated treatments of the soil by the chemical, would tend to make them progressively less effective against insects.

The experiments described here were designed to test these suggestions, and to attempt to determine the rate of decomposition of naphthalene in the soil. As a necessary preliminary, an investigation of methods for the determination of naphthalene in soil was undertaken.

The experimental work divides itself conveniently into two sections. The first part deals with the toxicity of naphthalene to insects in the soil, using wireworms as test subjects; and the second deals with the determination of naphthalene in soil and the investigation of its rate of decomposition.

The experiments were carried out in 1920 and 1921. Time was not available for their completion, but a number of points of interest emerged which it seemed advisable to put on record.

EXPERIMENTAL.

Pot and plot experiments on the toxicity of naphthalene to wireworms.

Method. Lots of 2000 gm. of soil which had been passed through a 3 mm. mesh sieve were thoroughly mixed with powdered naphthalene in quantities varying from 0·1 to 0·0125 per cent. and put into glazed pots of an internal diameter $5\frac{1}{2}$ in. and internal depth of $6\frac{1}{2}$ in. A number of wireworms (8–10) were placed on the surface and allowed to penetrate or in some cases were put in first at the bottom of the pot and covered by the soil. The pots were placed in a cellar which varied little in temperature from day to day and were covered with brown paper which was occasionally moistened to prevent undue loss of moisture from the soil. The level of the soil was usually $1\frac{1}{2}$ to 2 in. from the edge of the pot and the wireworms were unable to escape. The pots were examined at the end of one week.

At concentrations of 0·1 and 0·05 per cent. naphthalene was generally completely toxic and concentrations of 0·025 and 0·0125 per cent. were often lethal. The insects, however, varied in resistance to some extent according to the period of the year, and it is probable that just before moulting resistance is considerable, whereas immediately afterwards the wireworms appear more vulnerable to the action of the poison. As far as possible the insects chosen for experiment were of about the same size and in a state of activity; very light coloured individuals were rejected. In reading the data, it should be remembered that they refer to experiments carried out under conditions in which the insects had only a restricted area to move about in, and that they could not escape from the action of the poison, it is probable that in the open the results would have been somewhat less decisive.

The first set of experiments were designed to ascertain for how long a period naphthalene would retain its lethal properties in the soil. Different varieties of soil were chosen and, after putting through a 3 mm. sieve, treated with naphthalene in the usual way and examined from week to week till the toxic properties of the material were lost. A fresh batch of wireworms was added each week. The moisture content of the soil was determined from time to time and a little water added when necessary in an attempt to keep the moisture as constant as possible throughout the experiments.

The soils used were: (1) samples taken at different depths and a general sample to a depth of 9 in. from the manured and unmanured plots of Broadbalk field; (2) sample from Little Hoos field; (3) a richly

Table I.

Loss of toxicity with time of naphthalened soil (pot experiments).

(Controls with soil and with sand gave no deaths.) The soils were moistened occasionally.

(All figures are percentages.)

Naph. added	Soil		First week H_2O in soil	First week Wire-worms killed	Second week H_2O in soil	Second week Wire-worms killed	Third week H_2O in soil	Third week Wire-worms killed	Fourth week H_2O in soil	Fourth week Wire-worms killed	Fifth week H_2O in soil	Fifth week Wire-worms killed	Sixth week H_2O in soil	Sixth week Wire-worms killed
0·05	Broadbalk manured	1″	13·74	100	14·9	50	—	0						
,,	,, ,,	1–3″	11·6	,,	12·0	100	—	0						
,,	,, ,,	3–5″	13·0	,,	13·4	,,	—	0						
,,	,, ,,	3–7″	13·4	,,	12·8	90	10·85	100	—	0				
,,	,, ,,	7–9″	12·8	,,	13·1	100	13·6	,,	10·4	100	10·5	62·5	—	0
0·05	Broadbalk unmanured	1″	8·75	100	9·6	100	10·0	100	—	0				
,,	,, ,,	1–3″	8·6	,,	10·0	,,	9·9	,,	—	0				
,,	,, ,,	3–5″	9·3	,,	10·1	,,	10·7	60	—	0				
,,	,, ,,	5–7″	10·8	,,	11·6	,,	11·7	100	—	0				
,,	,, ,,	7–9″	13·5	,,	14·3	,,	14·9	,,	—	100	—	93	—	0
0·05	Broadbalk manured	1–9″	17·4	100	16·9	75	15·8	0						
0·025	,, ,,	,,	,,	,,	,,	0								
0·0125	,, ,,	,,	,,	80	,,	0								
0·005	,, ,,	,,	,,	12·5	,,	0								
0·05	Broadbalk unmanured	1–9″	10·9	100	10·2	100	9·2	100	10·2	37·5	—	0		
0·025	,, ,,	,,	,,	,,	,,	,,	,,	,,	,,	37·5	—	0		
0·0125	,, ,,	,,	,,	,,	,,	,,	,,	,,	,,					
0·005	,, ,,	,,	,,	,,	,,	0	,,	22	,,					
0·05	Little Hoos Field		11·43	100	12·3	100	—	0						
0·025	,,		,,	,,	,,	50	—	0						
0·0125	,,		,,	75	,,	0								
0·05	"Cucumber" soil		46·6	75	42·9	0								
0·025	,,		,,	25	,,	0								
0·0125	,,		,,	0										
0·05	"Allotment" soil		22·25	100	21·4	0								
0·025	,,		,,	,,	,,	0								
0·0125	,,		,,	90	,,	0								
0·05	"Allotment" soil + 15 % sand		17·8	100	17·8	0								
0·025	,, ,,		,,	,,	,,	0								
0·0125	,, ,,		,,	,,	,,	0								
0·05	"Allotment" soil + 15 % sand		15·8	100	15·8	0								
0·05	"Allotment" soil + 15 % sand (sterilised)		—	100	15·5	100	13·24	80	—	0				
0·05	Sand		1·24	100	0·75	100	1·66	100	—	100	—	0		

manured soil from a cucumberhouse; and (4) soil from an allotment in the laboratory grounds (a typical garden soil). The results are stated in Table I.

The data presented in Table I bring out several important points. It is clear, in the first place, that the effectiveness of the naphthalene depends on the type of soil: in the naphthalened "cucumber" soil all the wireworms were not killed at any of the concentrations tested (0·05–0·0125 per cent.), whereas with most of the other soils the higher concentrations were effective. Secondly, in well-manured soils, the naphthalene loses its toxic action more rapidly than in unmanured soils; thus, the naphthalened soil from Broadbalk manured plots (farmyard manure) only retains its toxicity for about a week or ten days, whereas in soil from the unmanured plot toxicity persists for three weeks. Thirdly, treated soils from different depths from the manured and unmanured plots of Broadbalk retain their toxicity for different lengths of time: the top inch of soil from the manured plot destroys the toxic action of naphthalene in a little over a week, the soil from the 2nd to the 5th inch in a fortnight, and from the 3rd to the 7th inch in three weeks, whereas in the soil from 7–9 in. deep—just below the depth of ploughing—the toxic action persists into the 5th week. Similar results were obtained with the soil from different depths of the unmanured plot, except that in this case the toxic properties of the naphthalened soils from the top to the 7th inch persist for approximately three weeks and in the soil from the 7th to the 9th inch until the 5th week.

The natural deduction to be drawn from these data appears to be that the loss of toxicity is due to bacterial action and this is confirmed by experiments with sand and with sterilised soil. A garden soil (known as "allotment" soil) was mixed with sand (15 per cent.) and autoclaved for four periods of three hours each; lots of 2000 gm. were then naphthalened under as sterile conditions as possible and placed in pots of the usual dimensions; wireworms were introduced and each pot covered with two sheets of brown paper. A similar experiment in which clean sand was used and a control with naphthalened unsterilised "allotment" soil with 15 per cent. of sand were set up concurrently. It was found impossible to keep soil sterile under these conditions for more than a short period, as wireworms themselves introduced a contamination factor; nevertheless, the unsterilised soil retained its toxicity for only a week, whereas in the case of the sterile soil treated with naphthalene toxicity persisted for from two to three weeks and in the sand for a full four weeks.

These experiments were carried out under conditions where finely divided naphthalene was thoroughly incorporated with the whole bulk of soil, earlier experiments having indicated that when naphthalene was mixed with the top layer of soil only, the wireworms generally migrated to the bottom of the pot, out of range of the toxic action of the naphthalene, and so largely escaped injury.

Another set of experiments was set up to test whether the degree of fineness of the naphthalene materially affected its toxicity or persistence in the soil. A dry soil containing 10 per cent. of moisture was chosen in which 0·05 per cent. of finely ground naphthalene was known to persist for two weeks, and it was found that when crystals of naphthalene of the size of a pea were thoroughly incorporated, toxicity persisted for three weeks, but that when the particles were less in size than this the toxic effects disappeared as rapidly as with the most finely ground material. Here it is probable that the toxic effects and their persistence were accentuated by the dryness of this soil, the moisture content falling as low as 8·6 per cent. in the course of three weeks. The data given in Table II afford evidence of this. In each case "allotment" soil with 15 per cent. of sand was used, and apart from the variation in the moisture content the soil samples were similar.

Table II.

Effect of moisture content of soil upon persistence of toxic action of naphthalene on wireworms.

		Percentage killed				
Moisture %	Naphthalene %	First week	Second week	Third week	Fourth week	Fifth week
18·1	0·05	100	0	—	—	—
,,	0·0375	90	0	—	—	—
,,	0·025	90	0	—	—	—
17·8	0·05	100	0	—	—	—
,,	0·025	100	0	—	—	—
15·8	0·05	100	0	—	—	—
10·8–8·6	0·05	100	100	80	23	0
,,	0·0375	95	100	0	—	—
,,	0·025	100	100	0	—	—
,,	0·05	100	70	33	0	—
,,	0·05	100	100	100	0	0

In considering the data in Table II, it should be realised that the resistance of the insects is undoubtedly affected by the dry conditions of the soil when the moisture content is 10 per cent. or below; nevertheless, these experiments afford clear evidence that the toxic action of naphthalene persists for a longer period under dry than under more

humid conditions of the soil, for the characteristic odour of the chemical disappeared from the moister soils in a period of about seven days, whereas in the drier soils it continued for some weeks. It is thus apparent that aridity imposes a limit upon the activity of the factor making for decomposition. It would be interesting to ascertain whether there be an upper limit to the range of water content of the soil above which naphthalene would be found to be relatively stable. This would possibly be the case, as free access of oxygen, which water-logging would prevent, would appear to be requisite for decomposition; it is, however, questionable whether the retardation would take place much below the water-saturation point and as the latter varies with the type of soil, the mere expression of the moisture values without reference to the soil type would afford no indication of the stability or otherwise of naphthalene in any particular soil.

By permission of Mr J. C. F. Fryer, an experiment on a larger scale was carried out in the grounds of the Plant Pathological Laboratory of the Ministry of Agriculture. Three plots of 1 square yard were divided off by corrugated sheeting sunk to a depth of 1 ft.; the soil to 12 in. deep in two plots was thoroughly mixed with naphthalene equivalent to 0·056 and 0·028 per cent. on the soil respectively, the third plot being left as a control. About 200 wireworms were placed in each plot and after a period of nine days the soil was gone through and the wireworms recounted. The central plot was subsequently treated with 0·019 per cent. of naphthalene.

I am greatly indebted to Mr E. H. Hodson, now of the Seale Hayne Agricultural College, for supervising these experiments and undertaking the laborious task of making the wireworm counts. The results are given in Table III.

Table III.

Small plot experiments on toxicity of naphthalene to wireworms.

(200 wireworms added to each plot.)

Soil treated to 1 ft. deep. Examination after 10 days.

Amount of naphthalene added	No. of wireworms recovered from each plot	No. unaffected	No. moribund
Control (untreated)	150	150	—
0·056 % (15 cwt/acre)	154	2	152
0·028 % (7·5 cwt/acre)	150	5	145
*0·019 % (5·0 cwt/acre)	167	86	81

* The wireworms in this plot found unaffected were put back, and the plot re-examined after a further seven days; 78 were found alive and 8 dead; after putting back the "unaffected" for another month no further deaths were noted.

Experiments on re-additions of naphthalene to soil.

If the disappearance of naphthalene from soil be due to bacterial action, the enhanced number of naphthalene-decomposing organisms resulting from the treatment should lead to a more rapid decomposition of doses subsequent to the first, with a consequent lowering of toxicity. Four small experiments were set up to test this view. Two lots of 500 gm. each of "allotment" soil with an addition of 15 per cent. of sand were treated with 0·05 per cent. and two with 0·025 per cent. of naphthalene and put in glass jars of about 600 c.c. capacity fitted with screw caps. Wireworms were introduced into each. After examination at the end of the first week the soils were re-treated with the same amounts respectively, a fresh supply of wireworms being introduced each week. The naphthalene from the two higher concentrations disappeared too slowly to give conclusive results, but the two experiments with the lower concentrations indicate that the second dose of naphthalene was less effective than the first. The data were as follows:

Naphthalene added 1st week %	Deaths 1st week %	Naphthalene added 2nd week %	Deaths 2nd week %
0·025	70	0·025	0
0·025	100	0·025	25

Loss of toxicity of naphthalene not due to volatilisation.

Experiments were set up in which naphthalened "allotment" soil and sand containing 15·4 per cent. moisture, was kept in glass jars closed with screw caps. The soil was examined each week and a fresh supply of wireworms introduced. After the first addition no further naphthalene was added. The results obtained are given in Table IV.

Table IV.

Duration of toxicity of naphthalene in closed vessels.

Percentage naphthalene in soil	Percentage deaths		
	First week	Second week	Third week
0·05	100	100	0
0·05	100	100	0
0·025	100	0	—
0·025	100	0	—
Controls	0	0	0

Reference to Tables I and IV shows that in the experiments carried out in open pots with a similar soil of about the same water content, the toxicity of naphthalene at concentrations of 0·05 and 0·025 per cent. does not persist beyond the first week, although in the closed vessels, naphthalene at concentrations of 0·025 per cent. disappears in this time, the higher concentration (0·05 per cent.) persists for a further week. The more rapid disappearance of the higher concentration in open pots would not appear to be due to volatilisation as the vapour pressure of naphthalene is very low, but it does point to the fact that either free access of oxygen is essential for the decomposition of the chemical, or that the factor making for decomposition is not so active in closed as in open vessels. The results obtained in closed vessels indicate that the loss of naphthalene from the soil is mainly due to some factor inherent in the soil rather than to volatilisation.

Experiments on the stabilisation of naphthalene in soil.

For the purpose of controlling pests or disease organisms in the soil by chemical treatment, it is essential that the chemical should persist in the soil sufficiently long for its toxic action to be complete; but not for so long a time as to be detrimental to the crop following the treatment.

Two methods suggest themselves as suitable for stabilising naphthalene:

(1) The incorporaton with the naphthalene of some other chemical or antiseptic.

Although not fully explored this method has so far not given very successful results.

(2) The substitution of some element or group in the naphthalene molecule.

Neither of these methods was fully investigated, but experiments with α-chlornaphthalene showed that the introduction of chlorine into the naphthalene ring had a marked stabilising influence. Lots of 500 gm. of "allotment" soil plus 15 per cent. of sand were taken and each treated with equimolecular quantities of naphthalene and α-chlornaphthalene. In addition, two sets were treated with mixtures of naphthalene and α-chlornaphthalene in equimolecular proportions. The treated soils were then placed together with a number of wireworms in glass jars, which were closed with screw-cap lids. Examinations were made each week when a fresh supply of wireworms was added. The results are given in Table V.

Table V.

Comparative duration of toxicity of naphthalene and α-chlornaphthalene in soil.

Treatment	%	Percentage deaths each week 1	2	3	4	5	6	7
Naphthalene	0·05	100	100	0	—	—	—	—
,,	0·05	100	100	0	—	—	—	—
,,	0·025	100	0	—	—	—	—	—
,,	0·025	100	0	—	—	—	—	—
α-Chlornaphthalene	0·064	100	100	100	100	100	100	0
,,	0·064	100	100	100	100	100	100	30
,,	0·032	100	100	66	75	0	0	—
,,	0·032	75	83	66	100	0	0	—
,,	0·032	100	100	80	75	50	0	—
Naphthalene and α-Chlornaphthalene mixture	0·025 } 0·032 }	100	66	0	0	—	—	—
,, ,,	,,	100	50	0	0	—	—	—
Control (1)	0	0	0	—	—	—	—	—
,, (2)	0	0	0	—	—	—	—	—

The results obtained with 0·032 per cent. of α-chlornaphthalene indicate that it is slower in its toxic action than naphthalene, but the data in Table V clearly demonstrate that it persists in the soil for a greater period of time. It is a matter of surprise to find that the mixture of naphthalene and α-chlornaphthalene fails in toxicity at the end of the second week and that instead of the α-chlornaphthalene exercising any stabilising action on the naphthalene, the latter tends to de-stabilise the chlornaphthalene. Although no opportunity presented itself of confirming this result, it will be shown later that the addition of naphthalene to the soil causes, after a few days, a considerable rise in bacterial numbers, and it is not unreasonable to believe that amongst the organisms selected out by the naphthalene and which presumably finally decompose it, there will be variations, and that amongst them certain varieties will be capable of breaking up the α-chlornaphthalene molecule; the presence of naphthalene in the soil will tend to increase their number and so lead to a more rapid break-up of the chlornaphthalene molecule.

Determination of the rate of disappearance of naphthalene from soil.

In view of the fact that the toxicity of naphthalene in the soil only lasted in many cases for a brief period, it was considered advisable to determine its rate of disappearance by chemical methods. Methods for the determination of naphthalene, particularly in coal-gas, have been described. These invariably depend upon the formation of a mole-

cular compound with picric acid, known as naphthalene picrate, $C_{10}H_8 : C_6H_3N_3O_7$, a relatively unstable body, but only slightly soluble in water. The reaction of naphthalene with picric acid takes place fairly readily, and the naphthalene can be determined either by direct weighing as naphthalene picrate or, if a standard solution of picric acid has been employed, by titrating the solution of picric acid before and after treatment with standard alkali[1]. Küster (6), Colman and Smith (1), Gair (2, 3) and Somerville (2) have devised different modes of carrying out the estimation of naphthalene by the employment of this reaction. Küster's method, as modified by Colman and Smith, appeared too complicated and too slow for determining small amounts at intervals of a few hours. The use of relatively large amounts of acetic acid in Gair's method added to the difficulties of estimating by titration with standard alkali. Somerville has suggested the employment of alcohol for the absorption of naphthalene and its subsequent precipitation by a large excess of an aqueous solution of picric acid; a modification of this method was finally adopted and is described on a later page, but in the earlier stages of the work a number of experiments were carried out using aqueous picric acid for purposes of absorption.

Estimation of naphthalene in soil. Two methods can be used for isolating naphthalene from the soil. (1) The naphthalened soil can be subjected to a stream of gas, at a moderately high temperature, the naphthalene vapour being absorbed in some suitable solvent or directly precipitated by a solution of picric acid. (2) The soil can be subjected to distillation with steam, and the naphthalene determined in the distillate. Both methods were used as a check upon each other.

(1) *Aeration method.* The naphthalened soil, after being mixed with sand, was aerated in a U-tube, the sand and soil being placed in the limb of the U-tube furthest from the absorption apparatus; the other limb contained glass-wool and above the glass-wool an amount of phosphoric pentoxide was loosely packed to absorb moisture and ammonia. Any carbon dioxide and sulphuretted hydrogen were absorbed by a caustic soda solution in a small all-glass absorber, which could be warmed if any lodgment of naphthalene was noted; the gases then passed by way of a tube, having several constrictions along its length and which acted as a spray trap, to two absorption vessels in series. The absorption vessels were tubular but gradually narrowed towards their lower ends so that the inlet tube only allowed the narrowest

[1] Titration of the picric acid or naphthalene picrate can also be carried out by means of titanous chloride (5).

of margins for the passage of air, a bulb mid-way along the tube permitting of a good deal of splashing without loss of solution. 4–6 c.c. of 0·9 per cent. aqueous picric acid were used for absorbing the naphthalene and at the end of the experiment the volume was brought up to a definite mark on the limb of the absorption vessel, by the addition of a little distilled water. After the experiment the precipitate of naphthalene picrate was centrifuged out and an aliquot part of the clear supernatant liquid was pipetted off and titrated with $N/50$ or $N/100$ caustic soda. A few experiments were carried out using a solution of picric acid in water containing 20 per cent. glycerine in the absorption vessel; the glycerine however was observed to introduce a buffering effect on the titration and was finally discarded. Air or naphthalene-free coal-gas, drawn through the apparatus by a water-pump, was used for carrying over the naphthalene. The use of coal-gas was finally discarded as the results obtained did not differ materially from those obtained with air. The U-tube was heated to 150° C. in a glycerine bath, and the whole absorption apparatus protected from draughts by means of an asbestos box, which could be kept at a fairly constant temperature by means of a micro-burner.

Acknowledgments and thanks are due to Major G. G. Hyde, for help given during the early stages of working out a suitable method of estimating naphthalene by the aeration method.

The titration of so highly coloured a solution as that of picric acid presented some difficulties—as in ordinary white light the turning point of the indicator was greatly obscured. It was observed, however, that if the titration were carried out in a light of about the same colour as that of picric acid solution, the latter appeared to the eye nearly colourless, and the end point was quite sharply defined. The titrations were carried out therefore in a room illuminated by means of an electric bulb immersed in a strong solution of picric acid; the addition of a little eosin to the latter rendered the effect of decolorisation still more marked. Brom-cresol purple, phenol or cresol-red were found to be the most suitable indicators.

The method is extremely tedious and requires continuous attention for several hours. It was finally replaced by a method depending on distillation in steam. Fairly constant results however were obtained and these are consistent among themselves and show the same relative order of differences as those obtained by a distillation method, to be described later.

Using 10 gm. of naphthalened garden soil containing 50 mg. of naphthalene per 100 gm. of soil for each test, eight estimations gave

results lying between 38·3 and 41·0 of naphthalene with a mean of 39·3 mg. At a later stage it was found that a greater proportion of the naphthalene could be recovered by using a smaller amount of soil in the tests and increasing the volume of the picric acid solution in the absorbers relative to the amount of naphthalene to be absorbed. The following results were obtained when 5 gm. of soil were aerated instead of 10.

	Added to 5 gm. of soil mg.	Recovered from 5 gm. of soil mg.
1	2·6	2·2
2	2·5	2·32
3	2·5	2·44
4	2·5	2·33
5	2·5	2·36

A control test with untreated soil showed neither concentration nor condensation in the absorbers and the titration figure of the picric acid was unchanged.

The formation of formaldehyde during the aeration of heated soil.

During the aeration in the cold of soil fairly rich in humic material, which had been heated to 150° C. for several hours and then allowed to cool, the characteristic odour of formaldehyde was noted. Its presence was confirmed by the phloroglucinol test. In this instance, lime was used in the U-tube and aeration was continued in the cold for much longer than usual. The reaction is of great interest in itself, but as formaldehyde affects picric acid solution, it also indicates that it is advisable not to prolong unduly the aeration of the soil after the naphthalene has been volatilised.

Experiments with a garden soil and with a soil from a cucumber house.

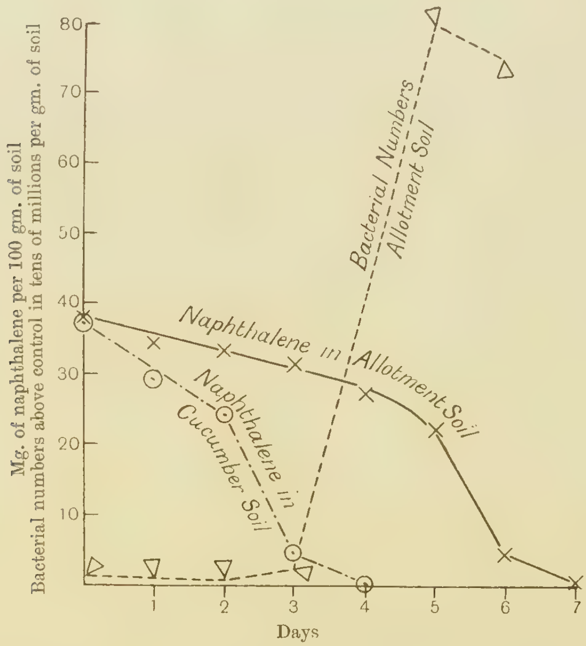
2000 gm. of a garden soil and 2000 gm. of a soil, rich in humus, from a cucumber house were each mixed with 1 gm. of finely ground naphthalene, placed in two large bottles, leaving ample air-space above the soil, and closed with cotton-wool plugs. Each day 10 gm. of soil were taken after thorough mixing and the naphthalene determined by the aeration method. At the same time the numbers of bacteria in the garden soil were estimated by Mrs D. J. Matthews, to whom the author wishes to express his thanks. The gelatine plate method was used in these counts. Later work has shown this method to give results of too high an order; absolute numbers are, however, for

Table VI.

Rate of disappearance of naphthalene from two soils.

10 gm. of soil used in aeration.

Day	Bacterial nos. in allotment soil, millions per gm. above control	"Allotment" soil. Naphthalene found in mg. per 100 gm. soil (1)	(2)	Mean	"Cucumber" soil. Naphthalene found in mg. per 100 gm. soil (1)	(2)	Mean
1	10	37·8	—	37·8	35·4	38·3	36·8
2	7·5	34·3	—	34·3	30	28·5	29·2
3	2·5	32·4	35·1	33·7	22	26	24
4	20·0	30·9	32·4	31·6	0·44	0·49	0·46
5	—	26·5	27·1	26·8		Trace	
6	800	21·2	23·4	22·3	—	—	—
7	750	4·2	3·9	4·0	—	—	—
8	—		Traces		—	—	—



×———————× Naphthalene found in "allotment" soil (aeration method)

⊙—·—·—⊙ ,, ,, "cucumber" ,, ,,

△- - - - - - -△ Bacterial numbers in "allotment" soil

Diagram 1. Decomposition of naphthalene in two soils.

this purpose of less value than relative figures, and the counts are of value and interest as they serve to show that a considerable rise in bacterial numbers took place just prior to and during the period when the rate of disappearance of the naphthalene was greatly accelerated. The data obtained are given in Table VI and the mean values are expressed graphically in Diagram 1.

These data indicate that the rate of disappearance of naphthalene depends entirely upon the type of soil used, the soil richest in organic matter and presumably therefore in microbiological population inducing the more rapid decomposition.

Decomposition in sealed bottles.

A further series of experiments was carried out with the "cucumber" soil. Eight lots of 400 gm. of cucumber soil were mixed with 0·2 gm. of powdered naphthalene, placed in separate bottles of 500-600 c.c. capacity and sealed by screw capped lids fitted with rubber bands. From time to time, the bottles were opened, the soil rapidly mixed, the bacterial numbers determined and the naphthalene estimated in 5 gm. of the soil, the remainder of the soil being put back into its bottle and sealed down.

The bacterial numbers were determined in triplicate by Mr D. W. Cutler, to whom the author wishes to express his thanks. The data are given in Table VII and Diagram 2.

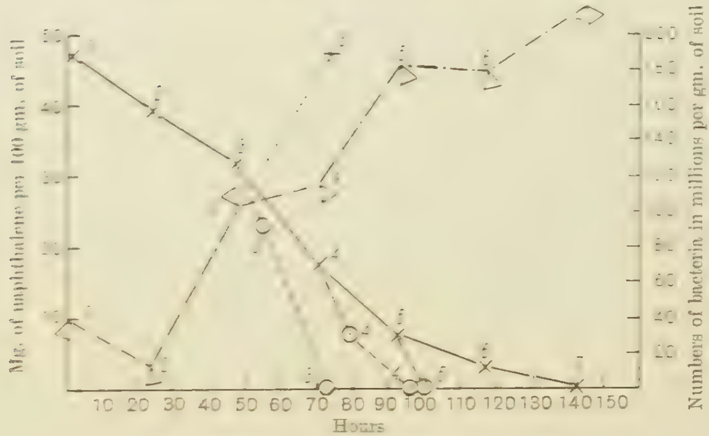
Table VII.

The disappearance of naphthalene from "cucumber" soil (sealed bottle experiment).

50 mg. naphthalene originally added per 100 gm. of soil.

No. of bottle	Time from mixing (hrs.)	First opening of bottle. Naphthalene found, mg. per 100 gm. soil 1	2	Mean	Bacterial nos. Millions per gm. soil. Mean of 3 determinations	Second and third openings of bottle: Time from mixing (hrs.)	Napthalene found, mg. per 100 gm. soil
1	0·5–0·75	46·6	47·1	46·8	39·3	5·3	43·3
2	23–24	39·6	38·0	38·8	14	29·5	36·4
3	47·5	32·4	31·6	32	102·6	55 73	(2nd) 22·8 (3rd) None found*
4	70–71	17·6	17·1	17·35	115·6	79·25 96	(2nd) 7·4 (3rd) None found
5	93–93·5	6·4	8·5	7·5	183·6	100·75	None found
6	117	3·2	—	3·2	179·3	124	None
	143	None	—	None	212	—	—

* Bacterial nos. rose to 194·3 millions per gm.



The numerals 1–7 are the numbers of the bottles

×———× Amounts of naphthalene at 1st sampling of each bottle

○- - - - -○ ,, ,, ,, 2nd and 3rd sampling of each bottle

△— — —△ Bacterial numbers at 1st sampling of each bottle

··········+ ,, ,, 2nd sampling of 3rd bottle

Diagram 2. The decomposition of naphthalene in "cucumber" soil (sealed bottles experiment).

It was noted that after any bottle had been opened, and the soil stirred, the rate of disappearance of the naphthalene was apparently accelerated, therefore, in addition to the determination of the naphthalene immediately after the first opening, the same bottle was reopened a few hours later, and again 24 hours after the first opening, and the amount of naphthalene again determined. An inspection of Table VII and Diagram 2 indicates that the opening of the bottle and the re-mixing of the soil materially expedites the disappearance of the naphthalene from the soil, and also causes a material increase in bacterial numbers. As little or no naphthalene could have volatilised in the brief time required for sampling, this simple operation must in some way have acted as a stimulus to the factor determining decomposition.

The preliminary fall in bacterial numbers indicates a selective toxic action of the naphthalene on part of the bacterial flora: the subsequent large rise shows that certain types of organisms are either capable of using naphthalene as a source of energy, or are stimulated to greater activity by its presence.

In all probability the estimations of naphthalene as carried out by the aeration method are valid in so far as they indicate the relative rates of disappearance of naphthalene from soil. The method as employed, however, is so tedious and difficulty to operate with success that it was considered advisable to check the results by another means. Somerville's method (2) indicates that alcohol can be used in the absorption vessels without interfering materially with the precipitation of naphthalene picrate, provided it is sufficiently dilute and a large excess of picric acid is present. It was therefore decided to use an alcoholic solution of picric acid for absorbing the naphthalene and to distil the latter from the soil into the alcoholic picric acid.

(2) *Estimation of naphthalene by steam distillation.* 100 gm. of naphthalened soil were acidified with the smallest amount of phosphoric acid necessary, and distilled in steam.

The outlet of the distillation flask was fitted with a spray trap through which the steam was passed into a Matthews ammonia absorption tube (7) containing 25 c.c. of a 5 per cent. alcoholic solution of picric acid, the absorption tube being allowed to get warmed by the passage of the steam in order to ensure complete reaction between the picric acid and naphthalene which distilled over very rapidly. A guard tube containing an aqueous picric acid solution was attached in series to the absorption tube to prevent loss of naphthalene. As soon as the greater portion of the naphthalene had passed over, both main absorption tube and guard tube were cooled. After the completion of the distillation, which took about half to three-quarters of an hour, the absorbers were aerated for 20-30 minutes while being cooled. Both absorbers were washed out into a 250 c.c. flask with an accurately measured quantity of aqueous picric acid solution and then with a little distilled water and the flask filled up to the mark. 100 c.c. of the liquid were then filtered and titrated with standard caustic soda. The titration was again carried out in a yellow or orange coloured light obtained in the way previously described. For amounts of naphthalene less than 10 mg. per 100 gm. of soil a small absorber was used and a correspondingly smaller amount of alcoholic picric acid (the alcohol present in the graduated flask should not exceed 10 per cent. in amount and should preferably be less). If H_2S or CO_2 is liberated in large amounts from the soil a second distillation flask containing caustic soda solution may be interposed between the first distillation flask and the absorber. This was not found necessary with the soil used and a control distillation of 100 gm. of the soil showed no effect upon the titration

of the picric acid. The following results were obtained in test trials:

	Naphthalene added to 100 gm. of soil mg.	Naphthalene found in 100 gm. of soil mg.
(1)	50	47·7
(2)	50	49·0
(3)	50	49·66
(4)	1·1	1·5
(5)	1·2	1·23
(7) Control	0	0

The method is less accurate for the determination of amounts of naphthalene less than 5 mg. per 100 gm. of soil than for the higher concentrations.

Experiments with repeated doses of naphthalene.

These experiments were set up to ascertain whether, when naphthalene had been added to the soil and allowed to disappear, and the soil again re-treated, subsequent doses would be decomposed more rapidly than the first, as would be expected if the decomposition were due to the micro-organic population in the soil.

Lots of 100 gm. of "cucumber" soil were therefore treated with 50 mg. of naphthalene and placed in 1000 c.c. flasks fitted with cotton-wool plugs. The rate of disappearance was determined and when the naphthalene content of the first series had been reduced to a minute amount (in 96 hours), a further quantity of 50 mg. was added to each of the remaining flasks and well mixed with the soil. The naphthalene now disappeared

Table VIII.

The effect of re-adding naphthalene to soil from which it had disappeared.

100 gm. of "cucumber" soil used for each test. Concentration of naphthalene at beginning 50 mg. per 100 gm. of soil.

First treatment		Second treatment		Third treatment	
Time	Naphthalene found (mg.)	Time (hrs.)	Naphthalene found (mg.)	Time (hrs.)	Naphthalene found (mg.)
4 hrs. 25 mins.	49·66	0	50 mg. (added)	0	50 mg. (added)
18 „ 50 „	47·34	24	1·0	3	20·2–21·2
45 „	36·76	—	—	5	8·7
72 „	2·7	—	—	8	2·8
77 „	1·0	—	—	11	2·3
96 „	Mere trace				

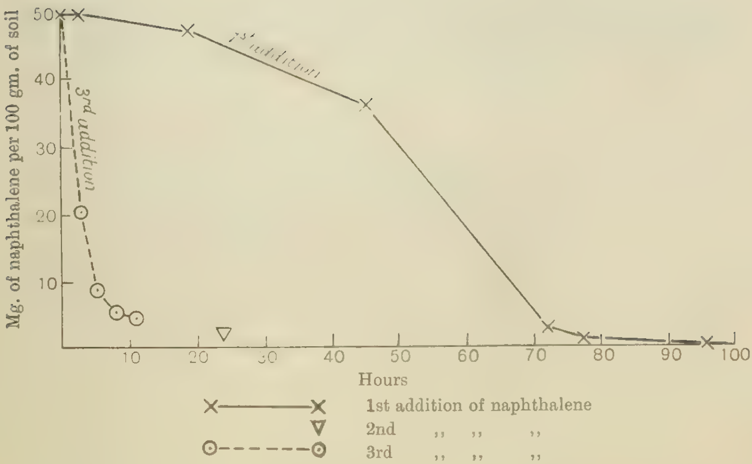


Diagram 3. Effect of re-adding naphthalene to soil ("cucumber" soil).

in 24 hours. A third addition of naphthalene was then made and at the end of three, five, eight and eleven hours, 20 c.c. of a solution of mercuric chloride was added in order to stop the reaction and the naphthalene remaining was estimated. The data which are set out in Table VIII and Diagram 3 clearly show that doses subsequent to the first one disappear from the soil at a much greater rate.

Experiments with sterile soil.

In view of the acceleration in the rate of decomposition of naphthalene on subsequent re-additions to naphthalened soil, experiments were made to test the rate of disappearance from sterile soil. Six lots of 100 gm. of soil from a cucumber house were placed in flasks of 1000 c.c. volume which were plugged with cotton-wool stoppers, so rolled that the flasks could just be supported when held by the wool. The soil was sterilised by autoclaving at 15–20 lb. pressure and then allowed to cool for 24 hours. Pure and sterile naphthalene was then prepared by flooding it in a round-bottomed flask with absolute alcohol, the alcohol being subsequently evaporated off *in vacuo*. 0·05 gm. lots (50 mg.) of the naphthalene were weighed out on sterile watch glasses and rapidly transferred to the flasks, well mixed with the soil and the flasks laid on their sides in a cellar which was known to keep at a fairly constant temperature for prolonged periods.

A similar set using unsterilised soil was set up at the same time. At intervals the napthalene in each flask was determined by the distillation method. The results are set out in Table IX and Diagram 4.

Table IX.

Decomposition of naphthalene and chlornaphthalene. Experiments with sterile and non-sterile soil (from cucumber house).

Time	Non-steril soil. Naphthalene found mg. per 100 gm. soil	Time	Sterile soil. Naphthalene found mg. per 100 gm. soil	Time (hrs.)	Non-sterile soil. Chlornaphthalene found. mg. per 100 gm. soil
—	50 (added)	—	50 (added)	—	0·05 c.c. (added)
4 hrs. 25 mins.	49·66	24 hrs. 10 mins.	49·55	—	50·1
18 ,, 50 ,,	47·34	96 ,,	43·8	47	48·4
45 ,,	36·76	240 ,,	43·4	78	45·0
72 ,,	2·7	—	—	172	41·7
77 ,,	1·0	—	—	214	42·8
96 ,,	Mere trace	—	—	362	33·4
				526	30·13

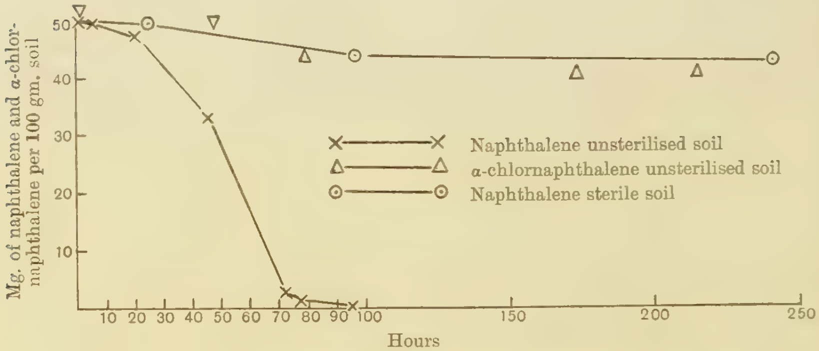


Diagram 4. Rate of disappearance of naphthalene and chlornaphthalene from "cucumber" soil

Inspection of Table IX and Diagram 4 confirms the deductions drawn from all the data given, that the disappearance of naphthalene is due to the micro-organic population of the soil. There is some loss of naphthalene from sterile soil, but as the cotton-wool plugs smelt slightly of the chemical, this was probably due to volatilisation.

Rate of disappearance of chlornaphthalene from soil.

In view of the rapid decomposition of naphthalene, experiments were made to ascertain whether it could be stabilised. It was considered that substitution of suitable groups in the naphthalene ring might be effective. Preliminary toxicity trials indicated that the substitution of a chlorine atom in the naphthalene molecule had a stabilising action (p. 66) and the rate of disappearance of α-chlornaphthalene from soil treated with this chemical was therefore determined. The experiments were carried out at the same time as the experiments set out in Table IX. Approximately 0·05 c.c. of redistilled α-chlornaphthalene was pipetted by means of a capillary pipette into each of six 1000 c.c. flasks containing 100 gm. of non-sterile "cucumber" soil, the flasks plugged with cotton-wool and laid on their sides in a cellar. From time to time the amount of chlornaphthalene was determined by distilling in steam into standard alcoholic picric acid as in the case of the determination of naphthalene. Control tests gave a recovery of (1) 51·1 and (2) 49·1 mg. of α-chlornaphthalene when the estimation was made shortly after mixing. The method is not quite as satisfactory for chlornaphthalene as for naphthalene; whether this is due to the chlornaphthalene not being quite pure or to a lower quantitative efficiency in its determination was not ascertained. The results, however, are comparable among themselves as the tests were carried out in as constant a way as possible. The data obtained are given in Table IX and Diagram 4. They indicate that there is a slight loss with time, but that chlornaphthalene is as stable in non-sterile soil as naphthalene in sterile soil, and while naphthalene disappears from "cucumber" soil almost entirely in 77 hours, chlornaphthalene shows only a comparatively slight diminution in amount in a period of 526 hours.

Nephelometric method of estimation.

Neither of the preceding methods could be regarded as suitable for estimating naphthalene in minute amounts. Some experiments were made to ascertain whether the nephelometer could be employed for this purpose, although these were not carried far enough to be employed upon naphthalened soil. It was found that by using a solution of picric acid containing 0·8 per cent. agar and 0·1 per cent. saponin and pouring in a solution of naphthalene in alcohol, the picrate of naphthalene was precipitated in a very finely divided form. The crystals, however, had a

tendency to grow or to aggregate, but this took place comparatively slowly. It is probable that some such method would be adaptable for tracing out the lower portion of the decomposition curves.

Erosion of a crystal of naphthalene.

An attempt was made to demonstrate the mode of attack upon a crystal of naphthalene. A modified Lipmann's medium was prepared containing naphthalene in place of dextrose, and 1 c.c. of a soil solution prepared from soil in which naphthalene had already been decomposed was pipetted into this medium and one subculture made in the same

Fig. 1. Erosion of naphthalene crystal (highly magnified).

medium. A small micro-petri dish was prepared by sealing by means of sodium silicate a small ring on to a microscope slide, and a slightly larger ring was sealed to a large cover slip. Both were sterilised and a few particles of recrystallised naphthalene were scattered on the cover-slip within the ring. One drop of the second subculture was allowed to fall on the naphthalene and a little melted nutrient agar was finally poured in, forming on cooling a thin semi-rigid film holding some of the naphthalene against the cover-slip; the cover-slip and ring were then placed over the ring on the microscope slide, allowed to stand at room temperature and examined microscopically each day. In three days

the edges of certain crystals showed very slight erosion. One of these was marked and after a week photographed. At the beginning of the experiment the edge *AB* was straight, but the illustration (Fig. 1) demonstrates that in a period of eight days it had been eroded into small bays. This serrating effect is probably due to unevenness in the decomposition of the crystal layers, which permitted attack at certain favourable situations along the crystal edges. In other cases erosion takes place more evenly but invariably bacteria were observed exhibiting strong Brownian movement in the medium close to the crystal edges.

All the evidence educed in the preceding pages points to one or more micro-organisms being the active agents in the decomposition of naphthalene in the soil. The biological aspects of the work were investigated by Gray and Thornton (4) who have demonstrated the wide geographical distribution of soils containing bacteria capable of using cyclic hydrocarbons, including naphthalene, as sources of energy. A number of these organisms have been isolated and described.

SUMMARY.

1. The insecticidal action of naphthalene and its duration in the soil have been studied.

2. When naphthalene is incorporated thoroughly with soil it shows a fairly potent toxic action on wireworms; uneven distribution lessens its efficiency as, owing to its low vapour pressure and consequent slow spread, it produces only a small zone of toxic action.

3. Naphthalene is slow in toxic action, taking three or four days to kill wireworms, as a consequence of which and of its repellent action to insects, if the chemical be unevenly distributed in the soil insects tend to move away from positions where toxic action would be exerted.

4. The persistence of the toxic action depends upon the soil type. In soils rich in organic matter, toxicity disappears more rapidly than in soils less rich in organic matter. Toxicity persists longer in sterile soils and in sand than in unsterilised soils, and in dry than in moist soils.

5. The rate of disappearance of naphthalene from soil has been determined. It depends very little upon volatilisation but almost entirely upon some factor inherent in the soil, which is more active in soils rich in organic matter than those poor in organic matter, and in unsterilised soils than in sterile soils.

6. Second and third doses of naphthalene added to the soil, when the first has disappeared, are decomposed more rapidly than the first dose.

7. The bacterial numbers of the soil are at first decreased by the addition of naphthalene, but there is a rapid rise during the period when acceleration in the rate of decomposition of the naphthalene is taking place. All the evidence indicates that the loss of naphthalene from the soil is mainly due to bacterial decomposition.

8. Experiments in sealed bottles indicate that the opening of the bottle and mixing of the sample expedite the disappearance of naphthalene from the soil.

9. The toxicity of α-chlornaphthalene persists for a longer time in soils than naphthalene, and is decomposed at a slower rate. An admixture of naphthalene appears to induce a more rapid disappearance of the toxicity of the chlornaphthalene. α-Chlornaphthalene is more toxic to plants than naphthalene.

10. Methods of estimating naphthalene are described. They depend on formation of naphthalene picrate. Picric acid can be more readily titrated by alkali in orange and yellow coloured light than in white light.

11. It was noted in several tests that the prolonged aeration of soils which had been heated and allowed to cool gave rise to formaldehyde.

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ON THE CONTROL OF RED SPIDER BY MEANS OF NAPHTHALENE VAPORISED OVER A SPECIAL LAMP

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(With 1 Text-figure.)

Red Spider, *Tetranychus telarius* L., has long been known as a pest of glasshouse plants, particularly cucumbers, vines and carnations; and within recent years it has become very prevalent on tomatoes. In the commercial cultivation of the latter crop in the Lea Valley and Guernsey Red Spider has spread to such an extent as to become a menace to the industry. Its ravages are well known and many methods have been tried for its control. Some growers still practise the method of vaporising sulphur from hot-water pipes, while others use sprays and dusts containing sulphur or sulphur compounds. No efficient control, however, can be claimed for these.

Within the last few years a considerable advance has been made by Speyer (1) who found that naphthalene vapour has a toxic effect upon Red Spider and its eggs. In 1923 he introduced the now well-known method of broadcasting No. 16 naphthalene. The naphthalene, which has been passed through a sieve of 16 meshes to the inch, is broadcast in cucumber houses at the rate of 3 lb. to every 100 ft. run of border, a minimum temperature of 74° F. and a high degree of humidity being maintained throughout the fumigation period of at least 12 hours.

While this method has proved effective for cucumbers it has certain disadvantages when applied to such glasshouse crops as carnations and tomatoes. Accordingly in 1926 the writer decided to attempt the vaporisation of No. 16 naphthalene by means of lamps.

The Vaporiser.

The first consideration was to secure a lamp at an economic price which would burn with safety for not less than 12 hours and generate enough heat to vaporise the required amount of naphthalene. Many types of lamps and fuel were tested and finally the paraffin lamp and

stand, as shown in the accompanying illustration, was designed. It consists of an oil reservoir of approximately 3 pints capacity, into which is fitted a single wick burner $1\frac{1}{2}$ in. wide, and surmounted with an aluminium chimney with a mica inspection window. A parabolic shaped

Fig. 1.

aluminium funnel fits round the top of the chimney, its function being to conserve the heat by deflecting it to the flat bottomed aluminium pan which fits into the top of the funnel. The pan will hold up to 5 lb. of No. 16 naphthalene.

Dosage.

The second problem was to determine the amount of naphthalene which could be used without injuring the plants and the best arrangement of the lamps in order to secure an even distribution of the vapour.

Experiments to ascertain effects upon plants.

No. of lamps	Time of fumigation	Duration (hours)	Concentration per 1000 cub. ft. (oz.)	Length of house (ft.)	Type of house	Temperature ° F. Min. Max.	Plants fumigated	Percentage moisture in house	Remarks	Observations	Percentage spider killed*
6	Night	12	2·5	200	Low ridge	75–80	Young cucumbers and tomatoes, in pots and borders. Carnations	Not known	Not damped down	Cucumbers scorched. Tomatoes in pots slightly scorched, those in border unaffected. Deposit of naphthalene on roof above lamps	90
6	,,	12	2·5	200	,,	75–80	,,	90	Damped down before fumigating	No scorch observed	90
6	,,	12	5	200	,,	75–80	,,	90	,,	Two month old plants in bloom unaffected, young seedlings in 60's slightly scorched	90
6	Day	12	10	200	,,	100	,,	90	,,	Two month old plants unaffected, also plants in border. Seedlings slightly affected	100
6	Night	12	15	200	,,	70–80	,,	90	,,	Two month old cucumbers and tomatoes unaffected, younger plants badly scorched. Plants in border still untouched by fumes	100

* Percentage kill of spider estimated.

Method of recording results in trial fumigations.

In recording the results the following methods were adopted. About one hour after the completion of the fumigation forty leaves were taken from plants in various parts of the house, placed in water and kept in a light and airy place at a temperature of about 60–65° F. On the third day each leaf was carefully examined and the number of active and inactive spiders recorded. As a control the same number of leaves were taken from the house prior to fumigation and examined in the same way.

Trial fumigations.

Warm, windless weather is best suited for the purposes of fumigation because there is less risk of draughts influencing the distribution of the fumigant and less likelihood of condensation taking place within the glasshouse owing to a fall in the temperature. Before fumigating, the capacity of the house was determined, and the house made as air-tight as possible, for leaks tend to lower the concentration of the gas. The plants in the house were thoroughly watered and the paths damped down, and the house was then closed for an hour or so before the fumigation in order to obtain a 90–95 per cent. relative humidity. Heat was applied when necessary to raise and maintain the glasshouse temperature at about 70° F., so that no condensation occurred during the time of fumigation. In the case of crops like tomatoes and cucumbers all ripe or almost ripe fruit was picked prior to applying the naphthalene to avoid its becoming tainted with the fumes.

The lamp wicks were cut level before lighting up in order to secure an evenly shaped flame free from peaks. Where lamps are being used for the first time it has been found advantageous to light them about an hour before actual use in order to ascertain if they are functioning properly. The lamps were then placed at intervals along the paths of the house to be fumigated in order to secure a uniform distribution of the vapour, and the naphthalene poured into the pans.

Fumigation of carnations. Carnations, being specially susceptible to Red Spider attack, were selected as a good subject for trial fumigations. Twelve fumigations were conducted at various dosages from 3·9–13 oz. per 1000 cubic feet. The temperatures varied from a maximum of 78–81° F. to a minimum of 60–70° F. and the relative humidity ranged between 80 and 95 per cent. In seven cases 100 per cent. kill was recorded and in the remaining five cases the percentage kill ranged from

90–97 per cent. Even with such low concentration as 4–5 oz. per 1000 cubic feet practical control was obtained. Full particulars of the fumigations are given below.

Table I.

Fumigations of carnations.

Concentration		House temperature			Period of fumigation	Condition of spider determined by counting		
Proposed oz.	Actual oz.	Max. ° F.	Min. ° F.	Humidity %	(hrs.)	Alive %	Dead %	Remarks
13	13	80	65	85	12	0	100	Slight scorch on some varieties
10	9·6	78	64	90	12	0	100	—
10	9·3	90	62	80	12	3	97	—
10	9·5	78	60	95	12	0	100	No damage to full blooms
8	7·5	70	63	90	12	0	100	—
8	8·0	82	70	95	12	0	100	—
8	8·0	70	63	—	13	0	100	Green fly and caterpillar also killed
6	6·0	95	62	95	13	0	100	—
5	4·5	90	63	85	12	4	96	—
5	5·0	85	65	85	12	6	94	—
4	4·0	70	63	90	13	10	90	—
4	3·9	80	60	90	12	7	93	—

Fumigation of tomatoes. In eight fumigations on tomatoes dosages ranging from 3·0–7·8 oz. per 1000 cubic feet were employed. The temperatures varied from a maximum of 74–99° F. to a minimum of 54–63° F., and the relative humidity from 84–95 per cent. In each case counts showed that 100 per cent. of the Red Spider were inactive after the fumigation, and in all dosages below 6·7 oz. per 1000 cubic feet no scorching of the foliage was evident 5 days after the fumigations. The particulars are given in the following table.

In addition to the two series of fumigations referred to above, forty-seven other fumigations were carried out on carnations and tomatoes in the Lea Valley, Middlesex, Worthing and Guernsey, with concentrations ranging from 6–13 oz. per 1000 cubic feet. In these cases the effect on the spider was only estimated, 100 per cent. kill being recorded when, after careful search, no living spiders could be detected, and 90 per cent. kill when in cases of heavy infestation only a few isolated spiders were found active after the fumigation. The results of these forty-seven fumigations were as follows: in 26 cases 100 per cent. kill was secured, and in the remaining 21 cases 90 per cent. kill resulted.

Table II.

Fumigations of tomatoes.

No. of lamps used	Proposed concentration per 1000 cub. ft. in oz.	Period of fumigation in hrs.	Actual concentration per 1000 cub. ft. in oz.	Temperature		Humidity %	Condition of spider determined by counting	
				Max. ° F.	Min. ° F.		Alive	Dead %
6	10·4	13	7·3	95	62	95	0	100
8	8·7	13	7·8	99	63	85	0	100
7	8·0	13	5·5	76	54	89	0	100
7	8·0	13	5·5	76	54	89	0	100
7	6·7	13½	3·5	82	61	89	0	100
7	6·7	13½	3·0	82	61	89	0	100
7	6·3	13	5·2	74	56	94	0	100
7	6·6	13	4·0	74	57	84	0	100

Fumigations have also been carried out on other plants at concentrations of 6–8 oz., and as shown elsewhere (2), vines, cucumbers, arums, smilax, *Asparagus sprengeri* and *A. plumosus nanus* have been successfully fumigated without showing any deleterious effects. In Worthing and Guernsey runner beans have also been successfully fumigated with naphthalene by this method.

Though the majority of the fumigations were carried out over night commencing about 5 p.m. some were conducted during the day time when weather conditions were dull and warm, in late spring and early autumn. The chief objection to daylight fumigations is the tendency for the temperature to rise too high, thus necessitating the opening of ventilators and a consequent reduction in the concentration of vapour.

It is evident that a wide range of plants can be successfully fumigated with naphthalene, for Hartzell (3) in America, using a slightly modified form of vaporiser and repeating the earlier experiments of the writer, extends the list of plants to forty species and secured practical control of the spider with 1·5 oz. per 1000 cubic feet. In addition he found that at this concentration *Heliothrips femoralis* and *Thrips tabaci* were also controlled.

Conclusions and Recommendations.

Vaporisation of naphthalene by means of lamps for the control of Red Spider has been carried out in various parts of England for the past 3 years, and evidence indicates that successful control is being obtained. The system is by no means perfected because of the important and variable influence of local conditions and further work is

advisable. One of the greatest difficulties in fumigating large blocks of houses such as exist in the Lea Valley is to secure an even distribution of the vapour throughout the block. Unfortunately, when the large block system of building was introduced little consideration was given to the practicability of successfully fumigating such large areas.

If Red Spider is to be successfully controlled in such types of houses it will be necessary to give consideration to the possibility of erecting portable screens so as to block off five or six large houses at a time. Damped Hessian screens hung from the gutters of the houses have been tried but this system of partitioning is clumsy and costly and by no means vapour-proof.

Each house has its own peculiarities, leakage, drift, soil conditions etc., factors which cannot be controlled. It is therefore necessary to take these into consideration when fumigating. It has been found that tomatoes and vines are more liable to damage than carnations and therefore greater care must be exercised when fumigating the former crops. When fumigating vines it has been found advantageous to spread a sheet of Hessian horizontally two or three feet above the lamp. This prevents a dense concentration of naphthalene immediately above the lamp. A crop badly infested with spider requires to be treated with greater caution than a normally healthy one owing to the lowered vitality of the plants.

The grower should be prepared to discover the best conditions for fumigating under his own local conditions. It is better to fumigate with low concentrations at frequent intervals rather than give one heavy fumigation. The effect on the plants is less likely to be detrimental if three fumigations are given with an interval of about one week between, than if the houses are fumigated only once using a very high concentration of naphthalene. It is best to start with a low dosage, gradually increasing it until satisfactory control is obtained.

Plants fumigated under dry conditions showed distinct signs of distress. It has been found that leaf scorch is more likely to occur in low-type houses, especially if the plants are in contact with the glass. Low night temperatures are also unfavourable for fumigations. They cause stratification of the vapours with the formation of pockets of concentrated vapour which are likely to induce scorching and may ultimately result in the condensation of the vapour. The grower must exercise judgment when conducting fumigations with naphthalene vapour, for there is always some possibility under certain conditions and with some species of plants of causing leaf scorch and general distress.

Although the fumes of naphthalene are not of a highly dangerous nature, it is imprudent to enter the house more often than is absolutely necessary during the fumigation. After the fumigation it is desirable that the fumes be quickly expelled from the house in order to maintain the plants in healthy condition. Plants which have been treated for spider invariably show the first beneficial results in the appearance of new growth, which is clean and healthy—in marked contrast with the old spider-ravaged leaves.

Cost of treatment.

The lamps herein described cost 14*s.* each when purchasing a dozen. Neglecting the cost of lamps, the cost of fumigating a house of 40,000 cubic feet capacity, using eight lamps and 20 lb. of naphthalene to get a concentration of 8 oz. per 1000 cubic feet, exclusive of time and labour is as follows:

12 pints paraffin @ 1*s.* per gallon	1*s.* 6*d.*
20 lb. naphthalene @ 18*s.* per cwt.	3*s.* 2½*d.*
	4*s.* 8½*d.*

This is equivalent to 1·4*d.* per 1000 cubic feet.

I am indebted to Capt. W. L. Henderson, Mr H. I. Kingston and Mr H. W. Miles for help in conducting the fumigations and assistance in various ways, and to Messrs Geo. Monro, Ltd., for permission to publish these observations.

Summary.

Naphthalene vapour having been shown by other investigators to be toxic to Red Spider, *Tetranychus telarius* L., it was thought that better control of the fumigation would be obtained if the substance was vaporised by means of a lamp. A suitable lamp was designed and is described herein. It has been used under commercial conditions and has given satisfactory results.

Carnations and tomatoes have been fumigated with naphthalene and satisfactory control of Red Spider has been secured as shown by data given herein.

Fumigations on a variety of glasshouse plants in addition to the foregoing have been conducted and general observations and recommendations on naphthalene fumigation for the control of Red Spider are included.

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ON THE LIFE-HISTORY OF "WIREWORMS" OF THE GENUS *AGRIOTES* ESCH.

PART IV.

By A. W. RYMER ROBERTS, M.A.
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(With 3 Text-figures.)

ON SPECIFIC CHARACTERS OF THE LARVA OF *AGRIOTES LINEATUS* L.

THE close similarity between the larvae of *Agriotes obscurus* and *A. lineatus* has for long been a difficulty with entomologists engaged in agricultural work and it therefore seems desirable that such information as has been obtained from a study of the exuviae. cast by a larva reared to the adult state, should be put on record, pending fuller information.

The larva in question, one of two given me by Mr F. R. Petherbridge from Huntingdonshire, was received in January 1922, when it was some 16 mm. in length. It was confined in a tin salve-box with moist soil and was fed upon potato placed in its box from time to time. Unfortunately, the two larvae were not kept separate and, as was to be expected, the smaller one soon disappeared. The one remaining was kept in the laboratory for the most part (though for some months its box was deposited in an open-air insectary) till the imago was found to have emerged on July 25th, 1925.

The distinction of the larva from *A. sputator* is of course a comparatively simple matter, but from *A. obscurus* it is more difficult. Beling [illegible], who had evidently bred the two species to maturity, if not from the egg, says of *A. obscurus* that the larva is almost entirely similar in appearance to that of *A. lineatus*, though it appears somewhat more strongly and thickly punctured and furrowed. In his synoptic table [illegible] he says also that the larva of *A. obscurus* is somewhat darker coloured. Henriksen [illegible] says that *A. obscurus* differs from *A. lineatus* in having more punctures, fewer and fainter furrows and by having a light longitudinal stripe on each side. Ford [illegible], after a scrutiny of Schiødte's figures [illegible], has suggested that the number of divisions in the anal pseudopod, the size of the subapical tooth of the mandible and the position

[illegible] of the head in *A. lineatus* differs in detail from that part in *A. obscurus*.

Taking these points *seriatim*:

1. *Sculpture and colour of cuticle.* My note on the appearance of this [illegible] are distinctly visible under the low power.

It is evident that the difference in the sculpture between *A. lineatus* and *A. obscurus* is not of the same order as that between either of these [illegible] distinction.

2. *The pale lateral stripe.* There is a rather broad area at each side [illegible] common to all healthy larvae of the genus.

3. [illegible]

flattening of the 9th sternite at the base of the pseudopodium. There appears to be no material difference between the two larvae in this respect.

4. *The mandible.* The flange-like denticle near the apex of the mandible appeared to Ford to be more pronounced in *A. obscurus* than in Schiödte's figure of the present species. A comparison of the mandibles cast by my specimen with a number of mandibles of *A. obscurus* fails, however, to reveal any great difference in this respect. As has already been pointed out (Part II, p. 204) the mandibles of wireworms are extremely variable, owing in great measure to the erosion to which they are subject. For this reason too it is unsafe to lay much stress on two other features which have been observed in the mandibles cast by this larva: (i) a distinct transverse suture extending from the base of the retinaculum (median denticle) to the outer margin of the mandible on the ventral surface, and (ii) the relatively greater length of the mandible, from the retinaculum to the apex, in the most perfect example, as compared with mandibles of *A. obscurus*.

The transverse suture mentioned above is found also in mandibles of *A. obscurus*, though it appears to be somewhat wider and more distinct in the specimens of *A. lineatus* examined. It exists too in *A. sputator* and apparently also in other members of this and other genera of the family *Elateridae*.

5. *Position of spiracle and setae in 8th abdominal segment.* Ford suggests that "the position of the spiracle and hairs on the 8th abdominal segment, as represented by Schiödte" fails to agree with the condition found in *A. obscurus*.

Comparison between specimens of the two species fails, however, to show any material difference in the position either of the spiracles or of the setae as between the two species. Schiödte's figure is drawn on rather a small scale and does not show all the setae present in the anterior row, adjoining the spiracle.

6. *Mandibular sclerites.* Henriksen says (p. 244) of the genus *Agriotes* that the lateral angles of the innermost portion of the mandibular sclerites project much further forward than the nasal teeth and his figure, representing the anterior portion of the head of *A. lineatus*, shows this clearly. In my specimens, however, the apices of the mandibular sclerites are quite blunt and but little produced beyond the nasale, resembling closely the figure of this region already given for *A. obscurus* (Part II, p. 205).

The *mouth parts* have been examined for distinctive characters, but apart from the rather unsatisfactory ones in the mandible, already referred to, nothing of much value has been found.

In the *antenna* the first segment has been found to be somewhat broader than that in the corresponding instar of *A. obscurus*. This difference is the most marked in the final instar, where it is of the order of 19 : 15. The second segment is also a little broader in the exuviae of *A. lineatus*.

But it is in the *spiracles* that the greatest difference has been found. In *A. lineatus* the thoracic spiracle is one-third longer than that of

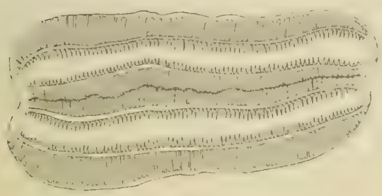

Fig. 1.

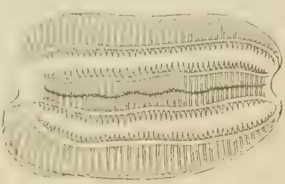

Fig. 2.

Fig. 3.

Thoracic spiracle of larva:—1. *Agriotes lineatus*; 2. *A. obscurus*; 3. *A. sputator*. Each magnified about × 280.

Fig. 1 represents the spiracle in penultimate instar, Figs. 2 and 3 in final instar.

A. obscurus, its breadth is slightly greater and it is noticeably more parallel-sided.

As is to be expected, the number of teeth at either side of the spiracular openings is also much greater in *A. lineatus*, counts of these amounting to 96 in the final and about 80 in the penultimate instar, against 47–50 in the thoracic spiracle of the final instar in *A. obscurus*. The abdominal spiracles resemble those of the thorax but are somewhat smaller. The number of teeth at each side of the respiratory orifice is about 75–80 in the first abdominal spiracle of the final instar, compared with 40–43 found in abdominal spiracles of *A. obscurus*.

In general appearance the spiracles resemble those of *A. sputator* rather than *A. obscurus*, but they are more parallel-sided even than these, have a much greater number of teeth bordering the respiratory orifice and are much larger in the corresponding instar.

Approximate measurements of the thoracic spiracle of the final larval instar are: length 0·212 mm., maximum breadth 0·101 mm.

Summary.

The most reliable characters for distinguishing the larva of *Agriotes lineatus* from the closely similar larva of *A. obscurus* have been found in the spiracles. The spiracles of *A. lineatus* are longer and more parallel-sided than those of *A. obscurus* and also bear a noticeably larger number of teeth bordering the orifices.

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THE GROWTH OF FUNGI IN SOIL[1]

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(With 1 Text-figure.)

I. Introduction.

It has long been recognised that the fungi are a normal constituent of the soil flora, but the condition in which they are present in the soil has been, and still is, a debated point. Waksman (12) devised a method which enabled him to state that some fungal forms, at least, exist in the soil in an active mycelial condition. He placed lumps of soil, procured under sterile conditions, on to plates of Czapek's agar. After an incubation period of 24 hours hyphae were found to radiate from the edge of the soil sample into the medium. The short incubation period excluded the possibility of spore origin for the mass of mycelium formed, for similar plates inoculated at the same time with spores alone, showed only minute colonies just discernible to the naked eye.

Brown (4) repeated these experiments and confirmed the results. Conn (6)—who advocates a direct microscopic examination of the soil for arriving at a quantitative estimation of the numbers of soil organisms—reported the almost entire absence of mould hyphae from soil smears examined by him and stained as directed with Rose Bengal in carbolic acid—a method primarily designed for the detection of bacteria in soil in this direct way. Later (1922) he modified the technique and substituted wet mounts instead of dry and used as a stain methylene blue, with the result that he could demonstrate fungus filaments in practically every soil he examined, but still they proved to be far from abundant.

Winogradsky (17) employs the direct microscopic method in his study of the soil and favours the view that the fungal constituents of the soil are present as spores, which become active, and therefore converted into the mycelial phase on the addition of an organic substance, *e.g.* cellulose, to the soil.

[1] From the Department of Mycology, Rothamsted Experimental Station, Harpenden, Herts.

Parallel with the above issue and to be considered alongside with it may be ranked the attempts made to determine the number of fungi in any soil (Waksman (13, 14) and Brierley (2)). The method most usually employed has been a dilution method, whereby a fraction of a soil suspension after suitable dilution is plated out in a series of petri dishes which are later poured with a cooled nutrient medium, incubated for 5–9 days and the number of fungal colonies appearing on each plate then counted. From this the number present in the original sample are calculated. The factors involved in this quantitative technique have been more recently studied by Brierley, Jewson and Brierley (3) of the Rothamsted Experimental Station and many of their methods have been adopted in the present investigation.

Conn (6) has pointed out that a large plate count may simply be due to the fact that an organism may have sporulated and may not necessarily indicate that fungi are playing any outstanding rôle in that particular soil. The following experiments are of interest in this connection.

II. Growth of pure cultures of fungi in sterilised soil.

Soil which had been sterilised by autoclaving was inoculated with equal numbers of spores of each of four different fungi, viz. *Alternaria humicola* Oud., *Penicillium lilacinum* Thom., *Trichoderma Koningi* Oud. and *Verticillium* (*Acrostalagmus*) *cinnabarinus* Corda, from cultures previously isolated by Brierley from Rothamsted soils. The number of spores per 1 c.c. of inoculum was determined by means of a Bürker haemocytometer. One c.c. of a suspension containing 3040 spores (760 of each genus) was added to 300 gm. of sterile soil, *i.e.* 10 spores for every gram of soil. This was kept at 9° C. together with an uninoculated control flask. At the same time triplicate plates of three different media (soil agar, Coons' agar and Conn's glycerine sodium-asparaginate agar) were inoculated in the centre with spores of the above fungi and incubated alongside the flasks. A temperature of 9° C. was chosen as convenient, for it delayed the sporing phase of some of the forms (see Fig. 1) and thus enabled one to compare the relative number of colonies of sporing and non-sporing forms which developed on the plates. The rate of growth of these forms in pure culture at this temperature was measured in terms of the increase in diameter of the colonies from day to day.

One week after the flasks had been inoculated, 10 gm. of soil were removed under sterile conditions and plates poured by the dilution method. Eight plates each for a range of dilutions from 1/80 to 1/20,480 were poured with the result set out in Table I.

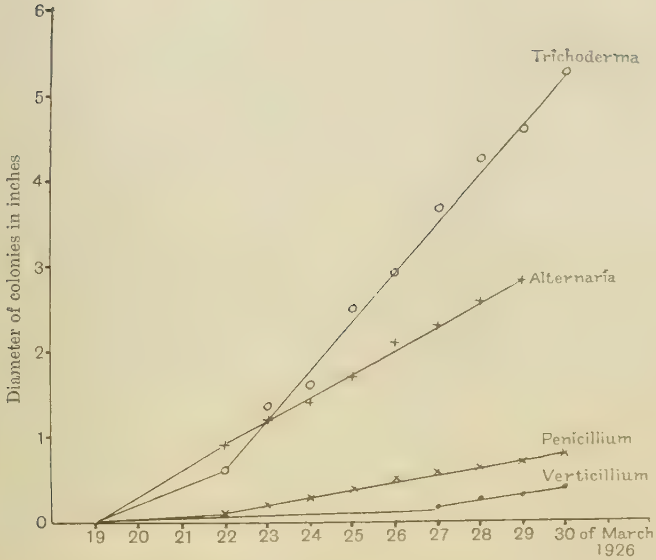

Fig. 1. The diameter represents the average of the growth on three media, viz. soil agar, Coon's agar and Conn's agar. Plates incubated at 9° C.

Table I.

The number of colonies obtained over a wide range of dilutions from a soil sample one week after inoculation with a mixture of spores from four different kinds of fungi.

Dilution	Average number of colonies per plate
1 in 80	40·37 ± 1·7
1 in 160	20·87 ± 1·7
1 in 320	9·43 ± 0·9
1 in 640	4·62 ± 0·8
1 in 1,280	3·12 ± 0·4
1 in 2,560	1·62 ± 0·4
1 in 5,120	0·50 ± 0·15
1 in 10,240	0·25 ± 0·13
1 in 20,480	0·12 ± 0·04

An analysis of the population showed that at the end of seven days the only form appearing on the plates was *Alternaria humicola*.

Reference to Fig. 1 shows that at this date both *Alternaria humicola* and *Trichoderma Koningi* in pure culture had approximately equal colony

Table II.

An analysis of the population appearing on the plates one week after the inoculation of the soil sample.

Plates ...	1				2				3				4			
Dilution	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*
1 in 80	45	.	.	.	36	.	.	.	42	.	.	.	39	.	.	.
1 in 160	16	.	.	.	19	.	.	.	16	.	.	.	28	.	.	.
1 in 320	10	.	.	.	11	.	.	.	6	.	.	.	13	.	.	.
1 in 640	4	.	.	.	3	.	.	.	6	.	.	.	2	.	.	.
1 in 1,280	3	.	.	.	3	.	.	.	3	.	.	.	5	.	.	.
1 in 2,560	.	.	.	.	2	.	.	.	2	.	.	.	1	.	.	.
1 in 5,120	1	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.
1 in 10,240	.	.	.	.	2	.	.	.	.	.	.	.	.	.	.	.
1 in 20,480	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	.

Plates ...	5				6				7				8			
Dilution	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*
1 in 80	44	.	.	.	47	.	.	.	34	.	.	.	36	.	.	.
1 in 160	24	.	.	.	17	.	.	.	23	.	.	.	24	.	.	.
1 in 320	7	.	.	.	Spoilt				9	.	.	.	10	.	.	.
1 in 640	8	.	.	.	4	.	.	.	8	.	.	.	2	.	.	.
1 in 1,280	3	.	.	.	5	.	.	.	1	.	.	.	2	.	.	.
1 in 2,560	.	.	.	.	3	.	.	.	2	.	.	.	3	.	.	.
1 in 5,120	1	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.
1 in 10,240	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
1 in 20,480	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

A = Alternaria humicola, P = Penicillium lilacinum, V = Verticillium cinnabarinus, T = Trichoderma Koningi.

Table III.

The number of colonies per plate eleven days after the inoculation of the soil sample.

Dilution	Average number of colonies per plate
1 in 80	Too crowded for accurate counts
1 in 160	
1 in 320	
1 in 640	132·75 ± 4·9
1 in 1,280	70·87 ± 0·88
1 in 2,560	38·25 ± 2·1
1 in 5,120	19·37 ± 1·15
1 in 10,240	12·5 ± 1·3
1 in 20,480	5·62 ± 0·78

diameters. *Alternaria*, however, was sporing whereas *Trichoderma* was entirely in the vegetative condition, and the mycelial development was apparently not sufficiently great to produce colonies with the amount of fragmentation induced and the dilutions used.

Eleven days after inoculation a further 10 gm. of soil were removed after thoroughly shaking the flasks, diluted through the same range and plated out as before (Tables III and IV).

Table IV.

Analysis of the population after eleven days of incubation of the soil sample.

Plates ...	1				2				3				4			
Dilution	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*
1 in 80	Rest	15	.	.	Rest	4	.	.	Rest	7	.	.	Rest	2	.	.
1 in 160	Rest	2	1	.	All	.	.	.	Rest	3	.	.	Rest	.	2	.
1 in 320	Rest	3	.	.	Rest	3	.	.	Rest	4	.	.	All	.	.	.
1 in 640	147	1	.	.	143	3	.	.	129	.	.	.	135	2	.	.
1 in 1,280	69	1	.	.	68	.	.	.	70	1	.	.	71	3	.	.
1 in 2,560	30	1	.	.	38	.	.	.	45	1	.	.	46	.	.	.
1 in 5,120	19	.	.	.	21	.	.	.	26	.	.	.	16	.	.	.
1 in 10,240	19	.	.	.	15	.	.	.	9	.	.	.	14	1	.	.
1 in 20,480	5	.	.	.	2	.	.	.	5	.	.	.	4	.	.	.

Plates ...	5				6				7				8			
Dilution	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*
1 in 80	Rest	11	.	.	Rest	3	.	.	Rest	10	1	.	Rest	8	.	.
1 in 160	Rest	5	.	.	Rest	4	1	.	Rest	5	.	.	Rest	7	.	.
1 in 320	All	.	.	.	Rest	2	.	.	Rest	3	1	.	Rest	2	.	.
1 in 640	119	1	.	.	109	1	.	.	125	1	.	.	144	2	.	.
1 in 1,280	70	1	.	.	74	1	.	.	69	1	.	.	67	1	.	.
1 in 2,560	32	.	.	.	42	.	.	.	32	.	.	.	39	.	.	.
1 in 5,120	16	1	.	.	17	.	.	.	18	.	.	.	21	.	.	.
1 in 10,240	9	1	.	.	12	.	.	.	7	.	.	.	13	.	.	.
1 in 20,480	8	.	.	.	7	.	.	.	5	.	.	.	9	.	.	.

The analysis again shows that the population of the plates was almost entirely *Alternaria humicola*, although the mycelial development of *Trichoderma Koningi* measured in terms of its colony diameter in pure culture is far in excess of that of *Alternaria*. In the lower dilutions a few colonies of *Penicillium lilacinum* appeared. Sporing in this case evidently occurred at about this time but was not evident to the eye in the pure cultures until the 13th day.

Twenty-seven days after inoculation 10 gm. were again removed and plated as before (Tables V and VI).

Table V.

Number of colonies per plate twenty-seven days after the inoculation of the soil sample.

Dilution	Average number of colonies per plate
1 in 1,280	Plates too crowded
1 in 2,560	Plates too crowded
1 in 5,120	103·87 ± 2
1 in 10,240	47·87 ± 0·9
1 in 20,480	29·62 ± 2·8

Table VI.

Analysis of the population twenty-seven days after inoculation.

Plates...	1				2				3				4			
Dilution	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*
1 in 1,280	Rest	124	4	2	Rest	111	5	2	Rest	134	2	2	Rest	109	1	2
1 in 2,560	Rest	79	6	1	Rest	63	6	2	Rest	98	9	2	Rest	84	7	3
1 in 5,120	50	43	4	2	54	44	6	2	52	40	6	2	50	50	4	.
1 in 10,240	21	27	3	1	18	28	2	.	25	20	3	2	20	25	2	1
1 in 20,480	16	17	3	.	10	10	4	.	14	17	1	.	11	11	2	.
Plates...	5				6				7				8			
Dilution	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*	*A*	*P*	*V*	*T*
1 in 1,280	Rest	153	4	2	Rest	130	6	1	Rest	135	4	3	Rest	124	5	2
1 in 2,560	Rest	71	10	1	Rest	92	7	1	Rest	90	7	.	Rest	70	8	2
1 in 5,120	51	39	6	2	53	45	4	1	53	56	5	2	52	49	9	.
1 in 10,240	19	25	1	.	23	25	.	.	20	22	1	.	29	18	2	.
1 in 20,480	12	13	1	1	16	12	.	.	13	23	2	.	13	14	1	.

At the end of twenty-seven days the numbers of *Penicillium* and *Alternaria* colonies were approximately equal to one another. This is due to the large number of *Penicillium* spores as opposed to the smaller number of large spores in *Alternaria* but reference to Fig. 1 shows the wide variance in the amount of mycelial development in the two cases.

These results show very clearly that the number of fungi occurring in soil, calculated by the dilution-plating method, can give no idea of the relative abundance or extent of the forms *actively* growing at any time in this substratum. Conn (6) attributed the paucity of fungal mycelium in soil as revealed by his technique to the fact that "fungus growth is not sufficiently abundant in that particular soil to show under the microscope." He further states that there is no real inconsistency between the high counts of fungi reported by Brierley (2), Waksman (14) and others, and his own small estimates, "for a colony of *Aspergillus*

growing on an agar plate bore spores enough to give a plate count of 300,000 per gm. provided they were distributed evenly throughout a kilo of soil and every spore was capable of growth, yet such a small amount of mycelium would be added to the soil that only one fragment of mould hyphae would be found in every 3000 microscopic fields."

The only conclusion one can draw from these statements is that Conn considers that the fungi are present in the soil largely in the spore state, and, so, high plate counts and feeble development of mycelium in soil, if this assumption is correct, are in no way contradictory.

III. Effect of drying under controlled conditions on the fungal population of the soil.

The questions then arise: "What is the condition of the fungi in the soil?" "Do spores predominate over vegetative mycelia?" and "What interpretation are we to place on plate count results?"

Waksman, as already pointed out, has demonstrated the fact that some active mycelium does occur in the soil, but apart from this there has been no attempt to try and measure the extent of this active phase or, indeed, to separate the active and spore members of the soil. In order to reach some conclusion in this matter the following experiments were performed.

A sample of soil was collected under sterile conditions and from it two representative samples of 10 gm. each were weighed out.

One of these 10 gm. samples was immediately placed in 100 c.c. of sterile water and vigorously shaken for half an hour. Fifty c.c. of this suspension was transferred by sterile pipettes to 50 c.c. of sterile water and so on until the desired dilution was reached. Sixteen 1 c.c. portions of the final dilution were then placed in petri dishes and the plates poured with Conn's glycerine sodium-asparaginate agar of a *p*H 4·7. The plates were incubated at room temperature and counts made of the colonies which developed.

The other sample was transferred to a sterile petri dish and placed in a desiccator over calcium chloride to dry. In order to hasten drying a vacuum desiccator was chosen and a cotton-wool plug was inserted between the pump and tap of the desiccator when the latter was disconnected from the pump. Air, before entering, had to filter through the plug and so the entrance of air-borne spores to the soil by the inrush of air was prevented.

The sample was left till dry; the usual time was three days, but drying was evident in a much shorter time. It was then removed from the

Table VII.

A comparison of the number of colonies obtained from an untreated soil sample and the number obtained from a similar sample that had been dried in a desiccator before plating.

Sample A. Dilution 1 in 20,480. Plates incubated for 10 days (counts made on the 3rd, 5th, 7th and 10th day).

	Untreated soil		Treated soil	
Plates	Total population	Fungal population	Total population	Fungal population
1	81	11	3	0
2	77	7	3	1
3	64	8	3	0
4	118	16	0	0
5	57	13	1	0
6	67	14	3	2
7	61	6	0	0
8	96	10	2	0
9	61	11	3	0
10	107	8	0	0
11	55	7	6	2
12	98	11	2	2
13	100	16	1	0
14	78	11	3	3
15	63	10	2	0
			3	0
Average	78·8 ±5·2	10·6 ±0·8	2·1 ±0·16	0·62 ±0·25

Table VIII.

Number of colonies obtained from a similar sample (see Table VII) which had been stored in the laboratory while desiccator sample was drying.

Plates	Total population	Fungal population
1	43	10
2	62	11
3	53	7
4	33	8
5	68	8
6	23	7
7	35	7
8	25	10
9	116	9
10	55	9
11	60	8
12	52	8
13	196	7
14	76	10
15	33	7
Average	62 ±11·3	8·4 ±0·3

desiccator and plated in identically the same way as the untreated sample, the same batch of medium being used in both cases. The results of the two platings were then compared (see Table VII).

As a check against this result a 10 gm. portion of the same soil sample was placed in a sterile container which was closed with a cotton-wool plug and kept in the laboratory for the same length of time as the sample was kept in the desiccator. This was plated out in the same way and at the same time as the latter. The results are shown in Table VIII.

As there is no significant difference between these results and those from the untreated sample, any biological changes taking place in the soil during the storage-period of three days does not account for the decrease in numbers obtained on plating out the treated sample.

The results for similar samples are given in the following tables.

Table IX.

Averages obtained from further samples subjected to the same treatment. Dilution 1/20,480.

Sample	Period of incubation (days)	No. of plates	Untreated soil: Total population	Untreated soil: Fungal population	Treated soil: Total population	Treated soil: Fungal population
B	8	16	33·7 ± 3·35	11·56 ± 0·84	4·43 ± 0·77	1 ± 0·18
C	12	16	163·7 ± 9·17	8·8 ± 0·74	84·5 ± 4·01	1·06 ± 0·30
D	9	8	142·5 ± 3·7	25·2 ± 1·8	126·75 ± 4·2	3 ± 0·71

As the fungal colonies were practically eliminated from the plates by this treatment when using a dilution of 1 in 20,480, *lower dilutions* were next used and parallel results were obtained, as shown in Table X.

Table X.

Sample	Period of incubation (days)	No. of plates	Fungal colonies*: Untreated soil	Fungal colonies*: Treated soil	Dilution
E	7	16	199 ± 4·37	13·7 ± 0·8	1 in 320
F	7	16	326·25 ± 18·15	5·56 ± 0·52	1 in 640

* These plates were examined microscopically and the fungal colonies counted in this way, for owing to the large number developing on the plates many did not reach macroscopic proportions.

In order to see if the reduced pressure was in any way responsible for the result in addition to the drying factor, a sample of soil was placed in the desiccator and allowed to dry over calcium chloride at ordinary air pressure. When dry it was plated out as before. The results are given in Table XI.

Table XI.

Number of colonies per plate from soil dried at air pressure.

	Untreated		Treated (dried at air pressure)	
Plates	Total population	Fungal population	Total population	Fungal population
1	162	9	74	0
2	180	13	55	4
3	135	11	88	0
4	191	7	90	0
5	115	4	62	1
6	172	10	68	1
7	184	7	76	1
8	166	8	110	2
9	198	9	86	1
10	137	10	69	2
11	144	9	116	0
12	170	8	107	4
13	155	11	72	1
14	186	6	93	1
15	193	14	68	2
16	206	9	77	5
Average	168·37 ± 6·07	9 ± 1·57	81·93 ± 4·41	1·56 ± 0·37

Experiments were then made to determine the length of time it was necessary to leave the soil sample in the desiccator to obtain a definite depression in the number of fungal colonies developing on the plates (Table XII).

Table XII.

Effect of varying periods of time in desiccator on number of colonies per plate.

Time in desiccator (hours)	Period of incubation (days)	No. of plates	Total population	Fungal population
0 (control)	7	16	196·5 ± 3·75	8·25 ± 0·465
4	7	16	174·43 ± 3·35	8 ± 0·24
18	7	16	121·8 ± 8·9	1·56 ± 0·29

Summarising these results, it is evident that when soil is dried in such a way as to exclude air contamination and then plated out by the dilution method the number of fungal colonies per plate is markedly decreased. In fact the whole population tends to show this depression; in some soils this is much more marked than in others.

Allison (1), when working on the biological changes in soil during storage, made a few determinations on the numbers of organisms in

air-dried soil as against the numbers present in a moist sample of the same soil. He found that air-drying caused a decided decrease in bacterial numbers in most cases, but the extent of the decrease seemed to depend largely on the kind of soil, a conclusion which is supported by the above figures. Too much reliance however should not be placed on the bacterial counts, for the acid medium used suppresses the development of many of the soil forms. He did not obtain any marked decrease for fungi, but this is not surprising as the soil was spread out to dry "in the open air of the dark incubating room."

These facts seem to indicate that the fungi are present in the soil almost exclusively in the *active mycelial condition* and when the soil is dried the hyphae are killed and therefore the low count results. That there is no induced formation of spores as a result of the subjection of the sample to drying is also evident from the low figures obtained. It may be argued that fungi inoculated into sterile soil not only grow luxuriantly but *spore* abundantly. However, soil which has been autoclaved is radically altered from a physical, a chemical and a biological standpoint. In its enriched condition, which is accompanied by a complete suppression of all biological competition, it serves simply as a good nutrient medium and consequently fungi inoculated into it run riot and carry out their normal life-cycle.

In order to obtain further evidence to support the contention, that the mycelial phase is all-important in the soil, a suspension of fragmented pieces of mycelia of *Trichoderma Koningi*, which had been grown in Richard's solution, was thoroughly shaken up with sand and also with sterilised soil. The resulting samples were placed in sterile petri dishes, transferred to the desiccator, the lids removed and left to dry out over calcium chloride and were finally plated out at a dilution of 1 in 640. A similar suspension was plated out directly at the same dilution. The number of colonies which developed per plate were compared in the two cases (Table XIII).

Table XIII.

Nos. of colonies	
Plated immediatel,	Plated after drying
20	1
18	0
21	1
25	2
16	0

Similarly, a suspension was made of the spores of four different fungi (*Penicillium lilacinum*, *Verticillium cinnabarinus*, *Trichoderma Koningi* and a green *Penicillium*) and 2 c.c. of this suspension was thoroughly mixed with 10 gm. of sterilised soil and placed in the desiccator to dry, after which it was plated out at a dilution of 1 in 2560. Two c.c. of the same suspension was then shaken in another 10 gm. of soil; this was plated out directly at the same dilution and the number of colonies per plate in each case were again compared (Table XIV).

Table XIV.

Nos. of colonies	
Plated immediately	Plated after drying
34	25
30	28
26	28
29	30
36	39

These results show clearly that drying of the soil samples would not prevent the subsequent germination of the fungal spores if present in the soil when plated out on a nutrient medium, whereas all the mycelial hyphae can be eliminated by this method and a comparison of the counts both before and after drying gives a measure of the activity of fungi in any particular soil. High plate counts, therefore, obtained by the dilution method suggest large mycelial development.

A further experiment was devised to test this point. Samples, each of 10 gm. of soil, were placed in a series of petri dishes and autoclaved at 20 lb. pressure for three periods of 20 mins. each. To each was then added 1 c.c. of a suspension of spores of three different species of soil fungi, viz. *Penicillium* sp. *Verticillium cinnabarinus* and *Macrosporium* sp. One sample was plated immediately on to Conn's glycerine sodium-asparaginate agar at a dilution of 1 in 20,480 (series A), a corresponding sample was placed immediately in a vacuum desiccator over calcium chloride and after two days similarly plated (series A_1). The remainder were incubated, one pair of samples for *three days*, the other for *six days* at 25° C. At the end of the third day, one was again plated at the same dilution without drying; a corresponding sample was placed in the desiccator for two days and then plated (series B and B_1). At the end of the sixth day one of the remaining samples was plated directly at a dilution of 1 in 655,360—this dilution was necessary owing to heavy sporing of the sample—the other was plated after drying (series C and C_1) (Table XV).

Table XV.

Sample	Period of incubation (days)	No. of plates	No. of colonies	Dilution
A	6	8	17·12 ± 1·1	1 in 20,480
A_1	6	8	13·5 ± 0·9	1 in 20,480
B	6	8	251·87 ± 11·3	1 in 20,480
B_1	6	8	149·12 ± 5·1	1 in 20,480
C	6	8	8·25 ± 0·5	1 in 655,360
C_1	6	8	5·37 ± 0·6	1 in 655,360

It will be noticed that the number of colonies developing on plates from sample B_1 is not so great as the number developing from sample B. An analysis of the colonies showed, however, that the proportion of colonies of each species remained approximately the same, pointing to the fact that the depression is due to the suppression of mycelium in the dried sample for, if spores were killed, the proportion would tend to be irregular (Table XVI).

Table XVI.

Sample B				Sample B_1			
		Analysis				Analysis	
Plates	Total	*Verticillium*	*Penicillium*	Plates	Total	*Verticillium*	*Penicillium*
1	228	19	Rest	1	155	10	Rest
2	239	40	,,	2	162	12	,,
3	318	30	,,	3	147	13	,,
4	244	27	,,	4	161	8	,,
5	285	25	,,	5	158	13	,,
6	233	38	,,	6	153	10	,,
7	241	35	,,	7	118	11	,,
8	227	20	,,	8	139	11	,,

It is interesting to note at this point that Ludwig (11), experimenting with cotton seed infected by the anthracnose fungus *Glomerella gossypii*, found that "storage in a very dry atmosphere, *e.g.* in a desiccator over calcium chloride, was found to prolong the life of the fungus to a great extent whatever the preceding treatment of the seed."

The fact that in no case were the colonies entirely suppressed by drying may be due to one or two factors. In the first place, as lack of time has necessarily made this work preliminary in character, no attempt was made to measure degree of drying and it may be that fragments of mycelium still retain their vitality at the end of the three-day drying period. More probably, however, as the surface layers were included in the original sample of soil used, some spores of air forms

may have been present: if so, these would withstand the drying period and appear on the plates.

The results arrived at by the direct microscopic examination of soil seem to be in conflict with the idea here suggested—that fungi are present in the soil extensively, in fact practically entirely, in the mycelial condition. However, it is now well established that algae and protozoa are present in soils in numbers far exceeding those estimated by direct microscopic examination. Cutler (8) has shown that the factors governing the relation between the protozoa and the soil particles are those of surface action. The same factors are no doubt operative in the case, at least, of the unicellular algae and the acknowledged difficulty in demonstrating their presence by the direct method, although they may be present in the soil in large numbers, is thus explained.

The fine fungal threads forming a network round the soil particles would, for similar reasons, be equally difficult to demonstrate in quantity and so no idea of the mycelial distribution can be gained by a direct soil examination.

Summary.

(1) Sterile soil was inoculated with a known quantity of spores of four different fungi, incubated at 9° C. and at intervals representative samples were plated out by the dilution method and an analysis of the plate population made. Results showed that high plate counts were not in any way connected with vegetative growth and supported Conn's idea that in such a case one is simply measuring the sporing capacity of the forms used.

(2) Samples of moist soil and of soil which had been dried in a vacuum desiccator over calcium chloride were plated out by the dilution method and the number of fungal colonies per plate compared. A marked decrease was noted with the dried sample. The reduced pressure was found to have no effect as drying under ordinary air-pressure gave comparable results.

(3) Suspensions in soil, and in sand, of fragmented mycelia and of a mixture of fungal spores, were in turn plated out directly and after drying. No colonies developed from the sample in the desiccator containing only mycelia, whereas the sample containing spores was in no way affected. It is suggested therefore that the decrease obtained after drying is due to the desiccation of the vegetative mycelium in the soil and since the reduction in the number of colonies per plate is very pro-

nounced after this treatment, it is thought that the normal fungal constituents of the soil are present extensively in the mycelial condition.

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THE EFFECT OF PHENOL, CARBON BISULPHIDE AND HEAT ON SOIL PROTOZOA

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Introduction.

Waksman and Starkey (11) found that when soil was treated with 1 per cent. carbon bisulphide the protozoa were absent after 14 days but reappeared at the end of 28 days. The disinfectant at first depressed the protozoa which later began to increase rapidly, reaching a maximum only after 90 days. When similar soil was reinoculated the rise in bacterial numbers was more rapid but this was followed by a rapid fall. The protozoa also were evident after 14 days. Russell and Hutchinson (7, 8) found that treating the soil with 0·5–1 per cent. carbon bisulphide produces an enormous increase in bacteria from 2–121 millions per gm. after a period of 74 days. Russell and Golding (6) in a previous paper gave similar results though the bacteria did not reach quite such high numbers. They believed that the vapour of carbon bisulphide has great power of penetrating the soil and of reaching organisms which other chemicals such as toluene leave untouched. Matthews (4) experimented with a large range of chemicals when working on the partial sterilisation of greenhouse soils and she found that the bacteria fluctuated, usually being reduced for the first few days and then rising to a maximum and gradually falling to normal. The whole process she says is much slower in field soil than in richer, lighter and better aerated greenhouse soil. Aeration she suggests has a great influence on the rapidity of these changes. She concluded that the rise in numbers of the bacteria is due not to the absence of the protozoa, but to the feeding effect of the antiseptic on the bacteria; and the increased fertility of the soil is to be attributed to the activity of the bacterial population in breaking down the organic matter of the soil. Sewertzoff (10), working on the influence of several antiseptics on soil amoebae and bacteria, gives results quite the reverse to those quoted above; she finds with carbon bisulphide that even such high dilutions as 20, 40 and 60 per cent.

are useless to kill off the cysts of the soil protozoa or the spores of *Bacillus subtilis*. Even a non-sporing bacterium as *Staphylococcus* was not killed with 10 per cent. carbon bisulphide. It must be remembered that though these high dilutions are not strong enough to kill off cystic protozoa, the active stages are readily killed.

Buddin (1), who worked at the partial sterilisation of soils by several antiseptics, found that phenol and its derivatives were effective if used in high enough strengths. He found that phenol in weak doses from $M/200$–$M/50$ causes a high rise in the numbers of bacteria, and that even up to 0·1 per cent. phenol there was no disappearance of the protozoa. Doses of $M/10$ to M kept the soil protozoa and bacteria in an inactive condition for 75 days. Buddin did not use carbon bisulphide in any of his experiments.

In view of these discrepant results it was decided to carry out experiments on the effects of phenol and carbon bisulphide on both the cysts and active forms of known species of protozoa. Further, as steam is extensively used as a partial sterilising agent in glass-house work and since its action may not be the same on the soil population as that of volatile antiseptics its effects were also tested. The species of soil protozoa used were: *Naegleria gruberi*, *Hartmanella hyalina*, *Oikomonas termo* and *Cercomonas crassicauda*.

METHODS.

The cysts of the protozoa used were tested in two ways to discover whether they were killed or not by the treatment with the disinfectant. First by cultural methods, *i.e.* by placing the cysts on agar plates or in hay infusion. Second by testing with 0·125 per cent. eosin.

This method has been used by Wenyon, O'Connor and Cutler (3) as a rapid method of detecting dead cysts of *Entamoeba histolytica*, where it was of great use.

Cutler (2) has also used it for soil protozoa, the cysts of which were boiled or heated at 85° C. for one hour. These cysts, when tested with the watery solution of eosin, became uniformly coloured, and when tested by cultural methods were found to be dead. Kessel (3), using this method for testing the viability of *Hartmanella hyalina* cysts when treated with chlorin water, finds also that all red cysts are incapable of development. Notwithstanding this the number of stained cysts does not in all cases represent the numbers of cysts which are killed. Frequently some of the cysts exhibit a condition of plasmolysis and have a yellow appearance. When the plasmolysis is complete, *i.e.* the cytoplasm

undergoes a very pronounced shrinkage, the cysts are then less likely to take the eosin stain. This complete plasmolysis was noticed occurring most commonly in high concentrations of chlorin water or in lower concentrations for extended periods of time.

Tests with *Hartmanella hyalina*, *Naegleria gruberi*, *Cercomonas crassicauda* and *Oikomonas termo* showed that the eosin method was effective with them, but with the flagellates *Helkesimastix faecicola*, *Heteromita globosa* and *Sainouron mikroteron* the dead cells were not coloured; in the case of the ciliates, *Colpoda steinii* and *C. cucullus*, the young or thin-walled cysts were coloured, but not all the older cysts with thick yellow and brown walls.

The eosin method is a useful rough test for the viability of cysts, but should be used in conjunction with other methods, and for some species of flagellates it is of no use. In all the following experiments, therefore, where the eosin method was used, the cysts were also plated on agar or placed in hay infusion to note whether excystation took place.

The effect of phenol and carbon bisulphide.

The following experiments were carried out to discover the strengths of phenol needed for destroying the active and cystic stages of the amoeba *Hartmanella hyalina* and two flagellates, *Cercomonas crassicauda* and *Oikomonas termo*.

Experiments 1.

Hay infusions containing the following strengths of phenol were used, 1, 0·5, 0·3, 0·15, 0·1, 0·055 per cent. and control.

Method. Cavity slides were filled with phenol hay infusion and active *Hartmanella hyalina* from a healthy rich culture were added. Evaporation was prevented by the slide being covered with blotting paper the central space being cut out, the paper was kept saturated with phenol hay infusion and placed in a damp chamber.

The amoebae in the dilutions from 0·1–1 per cent. were all killed; a few, rounded off with an unhealthy appearance, were found in the 0·055 per cent. dilution. The amoebae in the control were active and healthy. Another experiment was done in which active *Cercomonas crassicauda* were used instead of active amoebae. These were killed in every strength of the phenol while they remained healthy and active in the control.

Experiments 2.

The above experiments were followed by others in which cystic protozoa were used. As the form and results of the experiments are similar except for the protozoa used, it is needless to detail each in turn.

A suspension of cysts from an agar slope was made in 5 c.c. of sterile tap water, which was poured on to agar plates. The agar had previously been phenolised to the following strengths 1, 0·5, 0·3, 0·15, 0·1, 0·055 per cent. in duplicate and duplicate normal agar plates were used as a control.

The following cystic protozoa were used in different experiments: *Cercomonas crassicauda*, *Oikomonas termo* and *Naegleria gruberi*. In every case no excystation occurred in the dilutions above 0·3 per cent., though the cysts were not killed in the case of *Naegleria*, Table I; this result was doubly tested in the case of *Naegleria gruberi* as phenol hay infusion of the same strengths was used as well as the phenol agar.

Table I.

Days	1 %	0·5 %	0·3 %	0·15 %	0·1 %	0·055 %	Control
			Naegleria gruberi cysts plated on phenol agar.				
1	+	+	+	+	+	+	a +
3	+	+	+	a +	a +	a +	a +
4	+	+	+	a +	a +	a	a
7	+	+	+	a	a	a	a +
17	v f +	v f +	+	+	+	+	a +
			Oikomonas termo cysts in phenol hay infusion.				
2	+	+	+	a	a	a	a
5	+	+	+	f a	a	a	a
8	0	0	0	f a	a	a	a +
			Cercomonas crassicauda cysts in phenol hay infusion.				
1	0	+	+	+	+	+	a +
4	0	+	+	+	+	a +	a +
7	0	+	+	a +	a +	a +	a +
10	0	+	+	a +	a +	a +	a +

v f = very few, f = few, a = actives, + = live cysts, 0 = dead cysts or actives.

These results were again tested by two methods to discover whether the cysts were killed by the phenol: (1) by the eosin method, where the dead cells were stained pink; (2) by plating on normal agar plates and examining these at intervals of three days to note whether any active protozoa were present. *Naegleria gruberi* cysts under these tests gave no excystation on the phenol agar after 18 days above 0·3 per cent. phenol, but a few cysts were still living at 1 and 0·5 per cent. in the

phenol agar and hay infusion after the same period of time. The *Cercomonas crassicauda* cysts by the eosin test showed that a few cysts were still living after seven days in 0·5 per cent. phenol hay infusion, but the cysts did not excyst when placed on normal agar; while as stated above the cysts of *Oikomonas termo* were killed with 0·3 per cent. phenol agar. Thus, amoebae cysts in some cases may be depressed by the phenol and after a long period may resume their activity when the medium has recovered from the influence of the phenol.

Since it was shown by Sewertzoff (10) that the dosage of antiseptics required for soil was much larger than in the case of cultures, it was decided to test the strengths of phenol on the protozoa in soil.

These experiments were done on the untreated soil from a tomato house, where the soil fauna was well known. The soil was taken to the laboratory, sieved, and divided into six portions of 100 gm. each. Each 100 gm. of soil was placed in large petri dishes, and sprayed with the following strengths of phenol, 0·15, 0·3, 0·6, 0·9, 1·8 per cent., an untreated soil acted as a control. All strengths below 1·8 per cent. showed the presence of protozoa though in the case of the 0·9 per cent. dilution very few amoebae and flagellates were found.

Further experiments were made with the same soil treated with 1·2, 2·4, 3·6 per cent. of phenol. No protozoa were found in the 3·6 per cent. dilution, and very few in the two lower dilutions.

Two plots of soil in a tomato house were treated with 0·25 per cent. and 0·15 per cent. carbolic acid, the numbers of protozoa were not so low as after steaming but the depressing effect, particularly on the amoebae, continued for over 55 days in 0·15 per cent. carbolic.

This work showed that the strengths of phenol used in commercial practice are too low to kill the protozoan cysts, though they cause an immediate disappearance of active forms from the soil for long periods of time.

Buddin (1), who worked on the effect of partial sterilisation of soil by various antiseptics, found that $M/10$–M killed off the protozoa, $M/10$ being about equal to 1·2 per cent. which was used in these experiments. He found the same dose was necessary for cresol, which is one of the chief ingredients of the carbolic acid used in the above experiments.

The influence of carbon bisulphide on soil protozoa.

Experiments on the influence of carbon bisulphide on soil protozoa were carried out on the same lines as in the previous work on the influence of phenol.

The following dilutions of carbon bisulphide were made up with agar and hay infusion, 1, 0·76, 0·5, 0·3, 0·14, 0·06, 0·02 per cent. and control normal agar.

Experiments.

Method. Suspensions of cysts of *Hartmanella hyalina* were made in sterile tap water, 5 c.c. of which was inoculated into each of the following strengths (three plates for each), 1, 0·76, 0·5, 0·3, 0·14, 0·06, 0·02 per cent., and control.

A similar suspension of *Hartmanella hyalina* was inoculated into the same strengths of carbon bisulphide but hay infusion instead of agar was used, Table II illustrates the results both in the agar and hay infusion.

Table II.

The influence of carbon bisulphide on Hartmanella hyalina *cysts.*

	1 %		0·76 %		0·5 %		0·3 %	
Days	Agar	Hay	Agar	Hay	Agar	Hay	Agar	Hay
1	+	0	0	v f +	+	f +	+	+
3	+ a	0	+ a	0	+ a	f +	+	+
4	+ a	0	+ a	v f +	+ a	f +	+	+
9	+ a	0	+ a	0	+	0	+ a	+
	0·14 %		0·06 %		0·02 %		Control	
Days	Agar	Hay	Agar	Hay	Agar	Hay	Agar	Hay
1	+	+	+	+	+	+	+	+
3	+ a	+	+	+	+ a	+ a	+ a	+
4	+ a	+	+ a	+	+ a	+ a	a	+ a
9	+	+	+ a	+	+ a	+	a	+ a

+ = live cysts, a = actives, 0 = dead cysts or actives, v f = very few, f = few.

The experiments indicate that carbon bisulphide up to 1 per cent. in agar has no ill effect on cystic *Hartmanella hyalina*, which can excyst and continue to live in such agar; but carbon bisulphide in hay infusion has a decidedly depressing influence, a few cysts survived in 0·76 per cent. carbon bisulphide and lower dilutions for four days but no excysted actives were found above 0·02 per cent. after nine days.

Experiments were carried out on the influence of carbon bisulphide on active *Hartmanella hyalina* in cavity slips as above and kept in a damp chamber. The same range of dilutions were used as in the earlier experiments, but the first result of the treatment was to cause the *Hartmanella hyalina* to encyst, not to die. After three days, dead cysts

were found in the three higher dilutions, *i.e.* above 0·3 per cent., the same results continuing to the end of the experiment after fifteen days. The control had actives and cysts during the whole of the experiment.

The influence of carbon bisulphide on *Cercomonas crassicauda* cysts was studied, the dilutions 1, 0·76, 0·5, 0·3, 0·14, 0·06, 0·02 per cent. were used with hay infusion. It was found that at 0·5 per cent. and above the cysts were still found at the end of eleven days, while at 0·3 per cent. and below active *Cercomonas crassicauda* were found. In a similar experiment done on agar the results were similar.

When active *Cercomonas crassicauda* were treated with the same strengths of carbon bisulphide in hay infusion, in every case, except in the control, the *Cercomonas crassicauda* were killed off.

The experiments also show that the active forms of *Hartmanella hyalina* and *Cercomonas crassicauda* have a great difference in their tolerance of carbon bisulphide. Unless strong doses of carbon bisulphide are used for *Hartmanella hyalina*, the effect is to cause the actives to encyst and when the chemical has partially evaporated to excyst again, whereas *Cercomonas crassicauda* is killed.

The effect of heat.

Soil heated to 60° C. was found by Russell and Golding (5, 6) to give a great increase in the number of bacteria but on the whole not such high numbers as when similar samples were treated with carbon bisulphide.

The same results, high numbers of bacteria, were again obtained by Russell and Petherbridge (9) in further experiments of heating the soil to 98° C. The effect was not found however when soil was heated to 50° C. or 55° C., the numbers in every case falling below those of the untreated soil. The soil in this case was kept at 55° C. for three hours. Heating soil to various temperatures was done by Russell and Hutchinson (7, 8) to find what temperature was required to kill off the detrimental factor, *i.e.* the protozoa in the soil. Various temperatures of 40° C. up to 65° C. were used, and the results showed that heating to 55° C. did not extinguish the factor but that in another soil heating at 50° C. for twelve hours temporarily extinguished the protozoa. 65° C. appeared to be ample, though the rise in numbers from 3 millions per gm. of soil to 60 millions in 210 days was not a very great increase.

Methods.

Cysts of *Naegleria gruberi* were placed in a test-tube containing sterile water, which was then placed in a container having water at the required temperature and kept there for a specified time. The viability of the cysts was then tested by the eosin method and by cultures.

Experiments.

The percentages killed as tested by eosin were: at 45° C. approximately 45 per cent., at 55° C. 71 per cent., at 65° C. 90 per cent., at 70° C. 98 per cent. or sometimes 100 per cent. It was thought that as the temperature of 70° C. gave such a high death rate, 75° C. would be a suitable temperature to test the amount of time necessary for the cysts under similar conditions to be exposed. Thus the experiment was repeated on the following day with cysts from the same culture, and with a mixed collection of cysts from several cultures heated to 75° C. for five and ten minutes.

The results with the eosin test gave 93 per cent. killed at five minutes and 100 per cent. killed at ten minutes for all cysts.

As a result of this experiment several others were done with sieved soil from the farm-yard manured plot from Barnfield. These showed that a temperature of 85° C. for fifteen minutes or longer is sufficient to kill off all the protozoa in 5 gm. of soil in a test-tube.

Another experiment with approximately 500 gm. of soil was carried out where light soil was sieved and divided into four portions, three of which were steamed for three different periods of time, seven, fifteen and thirty minutes, one being reserved as a control.

In the case of that heated for thirty minutes, no trace of any protozoa was found during the twenty-nine days that the soil was examined; in the soil heated for fifteen minutes a few flagellates and amoebae were occasionally found. The ciliates appear to be very susceptible to heat, as the actives and cysts were killed off after seven minutes in the steamer.

A further experiment, however, showed that thirty minutes at 99 °C. is not absolutely lethal to all protozoa as both *Cercomonas crassicauda* and *Heteromita globosus* were present in small numbers in some further soil treated in that way.

These experiments show that a temperature of 99° C. is usually efficient in partially sterilising the soil if the heat is continued for thirty minutes. In one case when the soil was kept in the steamer for fifteen minutes after the steam had risen almost all the protozoa were killed,

a few flagellates being found on three samplings and amoebae twice out of twenty-nine samplings. Probably the physical conditions of the soil, such as the moisture content, will have an influence on the heat necessary for partial sterilisation.

Steaming experiments on tomato soil were done for two successive years. The temperature in the first year reached 100–105° C. at the edge of the plot, the steam being kept at the same pressure for one hour. The temperature at 9 in. after the steam was turned off was 98° C. The result of this treatment was to destroy almost all the bacteria and protozoa, though in one soil sample no protozoa were found. The protozoa numbers after twelve days were very depressed and were still low twenty-seven days after and continued depressed for some time. The steaming of the plots for the second year was not so drastic, the steam being continued for thirty minutes but the temperature did not rise above 60° C. This steaming reduced the numbers of the protozoa and bacteria so little that one of the plots was steamed a second time, the temperature rising on this occasion to 100° C. The protozoa, particularly in the twice steamed plot, showed a depression in numbers for 120 days after the steaming. As this lethal effect was too prolonged to be due only to the effect of steaming, a soil extract from the steamed soil was made, which proved lethal to active amoebae, though a similar extract from the untreated plot had no such effect.

Summary.

(1) Experiments made to discover the death-point of protozoa by phenol, carbon bisulphide and heat showed that phenol has a greater lethal effect than carbon bisulphide.

(2) Heating the soil in a steamer for thirty minutes is usually sufficient to kill off the protozoa, and the treatment of glass-house soil by steam destroys the majority of the protozoa and has a depressing effect on their numbers for a long period.

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PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

Ordinary Meeting held at 2.30 p.m. on October 28th, in the Imperial College of Science. The President, Mr J. C. F. Fryer, M.A., in the Chair.

Agriculture in Tropical Africa.

I. "Planting Developments and Difficulties in Nyasaland" by Dr E. J. Butler, C.I.E., F.R.S., Director of the Imperial Bureau of Mycology.

II. "The Work of the Amani Institute" by W. Nowell, D.I.C., F.L.S., Director of the Amani Research Institute, Tanganyika Territory.

II. THE WORK OF THE AMANI INSTITUTE.

By W. NOWELL, D.I.C., F.L.S.

The Amani Research Institute is situated towards the north-east corner of Tanganyika Territory, 5 degrees south of the equator and 38 degrees east of Greenwich. This position lies in the eastern section of the Usambara mountains, which rise abruptly from the level of the Pangani valley on its northern side and are continued by the western Usambara and the Paré mountains to the neighbourhood of Kilimanjaro. The buildings occupy the crown of a series of convergent ridges at a height of 3000 ft. and the cultivation extends down the slopes to a mountain stream at 1300 ft. and ascends a neighbouring summit to a height of 3700 ft.

The region is one of heavy rain forest of the usual mixed tropical type and the ground flora is of the soft and luxuriant nature associated with shelter and high humidity. Maidenhair fern and balsams may be mentioned as characteristic plants.

The nearest port is Tanga, distant by road some 50 miles. For half this distance, to Muhesa, the Tanga-Moshi railway is available. The branch line marked on the maps is no longer working. There is a coast road connection *via* Tanga which enables Mombasa to be reached within 24 hours in favourable seasons. The main line railway previously mentioned connects through Moshi and Voi with the Kenya-Uganda railway system and its steamers on Lake Victoria. There is a dry weather road, very good for most of the way, between Korogwe on the northern line and Kilosa on the Central railway from Dar-es-Salaam to Lake Tanganyika. This road continues south through the Tanganyika highlands to the head of Lake Nyasa or through Abercorn on to Broken Hill on the Northern Rhodesian Railway. For more than half the year there is access to the south only by way of the sea.

The climate of Amani is illustrated graphically in the exhibited chart for 1926 prepared by Mr C. B. Williams. The average rainfall is in the neighbourhood of

75 in. with recorded extremes of 55 and 95. The annual mean of atmospheric humidity is 86 mb., the annual mean temperature 67·8° F., the mean daily maximum 76·8 and minimum 63·7° F. The weather is for the most part cool and pleasant but with the prevalence of cloud and mist in the rainy season it is dank, not to say dismal, at times and for this reason a wood fire in the evenings is a comfort.

The area of the station is 750 acres, of which about a third remains under the original forest. A large part is occupied by permanent plantations. There are several small areas under Robusta and Arabica coffee and one in tea. There is a considerable area under Cinchona and there are plots of varying size of the different rubber trees, camphor, cinnamon, oil-palm, shade trees (*Erythrina*, *Grevillea*, *Gliricidia*), and numerous introduced timber trees including *Eucalyptus* spp., *Cedrela* spp., teak, and several of the tropical or sub-tropical Coniferae. There is a fine collection of bamboos, and a large and miscellaneous collection of trees yielding fruit or economic products of one sort or another. The collections of less durable plants have naturally for the most part disappeared. There is a large pasture in which *Paspalum dilatatum* from Brazil has been most successfully established.

Fortunately for the prospects of extended work at Amani there is now available, through the foresight of Mr Ormsby Gore and the consideration shown by the Tanganyika Government, the large neighbouring estate of Kwamkoro, the former property of Prince Albrecht, which contains large clearings under Arabica coffee, a well-equipped factory, and considerable areas of level ground suitable for arable cultivation. Possession of this estate will give the Institute a large-scale contact with practical agriculture which cannot fail to be valuable.

It was found necessary in German times, and will be again, to establish sub-stations for work on the crops of lower and higher altitudes. Owing to the situation of Amani the levels with fully tropical conditions, in which such plants as coco-nuts, sisal, and cotton are grown, can be reached at no great distance, while the neighbourhood of Moshi and Arusha provides conditions representative of the inland plateaux. The contemplated sub-stations may be either temporary or permanent according to the purpose for which they are established.

The *Biologisch-Landwirtschaftlichen Institut* was established in 1902 by the Government of German East Africa. It was laid down in the original decree that the work of the Institute was to be directed to the practical needs of the colony. The Institute was not to concern itself with scientific studies which did not contribute to the maintenance and improvement of East African agriculture. That these instructions were not meant to be interpreted in a narrow sense is shown by the inclusion in the programme of work, after the list of more obvious duties, of the investigation of the fauna and flora of the country.

Dr A. Zimmermann was the first (Acting) Director. Dr F. Stuhlmann was Director-in-charge for three years from 1905, after which Dr Zimmermann resumed charge and continued until the British occupation. A summary of the work of the Institute up to March 1914 may be found in *Beiheft zum Pflanzer*, vol. x, No. 3. The permanent staff when developed appears to have consisted of two chemists, two botanists, and a zoologist, with the additional services of three other men of science for varying periods, and in addition a European laboratory, office, and garden staff. The buildings were substantially constructed of stone and comprised a library, a botanical laboratory and herbarium, a zoological laboratory, a chemical building

with a well-equipped industrial section, numerous sheds and workshops, eight staff residences, and some smaller houses for minor officers. There was also a hostel for the accommodation of visitors, a post and telegraph office, a dispensary, a school, and a considerable native village for the labourers.

The scientific work carried out at Amani during the German times is recorded in ten volumes of *Der Pflanzer* and is of the quality and content one would expect from a highly scientific and industrious race brought into contact with agriculture carried on under a climate and conditions and with crops of which it had little or no previous experience. The extent to which Amani was able to be of service to the German Colonial Army during the war, a reference to which may be found in the report of the recent East Africa Commission, shows what excellent use had been made of the opportunities for the study of the manufacture of tropical plant products from quinine to a particularly potent brand of whisky.

The greatest success of Amani is probably to be found in its work of plant introduction. The thriving sisal industry of East Africa is the most vigorous of its offspring, and, but for a piece of ill-luck in backing the wrong horse, namely Ceara instead of Para, there might have been a flourishing rubber industry. The handling of the coffee plantations, if one may fairly judge from present indications, showed that the assiduous collection of information cannot take the place of practical experience gained in contact with tropical conditions.

After the termination of the war an attempt was made to continue the Amani Institute as a sub-department of the Tanganyika Department of Agriculture, and Mr A. Leechman served as Director from 1920 to 1923, when the attempt, which does not seem to have been more than half-hearted, was given up. The Institute was left in charge of Mr F. M. Rogers, a student gardener from Kew, who, under the control of the Director of Agriculture, functioned as curator up to March this year. Sufficient funds were provided by the Tanganyika Government to keep the grounds in order, to preserve the books, collections and apparatus and to maintain the function of plant and seed distribution. Reports have been current which suggested the looting of the Institute during the war, and neglect amounting to ruin in more recent years. I was agreeably surprised to find the cultivations in excellent order, and the books, most of the apparatus, and the collections in a condition which speaks well for the preservative effects of the Amani climate as compared with that of other tropical countries with which I am familiar. Mr Rogers has earned great credit for the thoroughness with which he has carried out his duties as custodian under conditions of isolation which would have damped the enthusiasm of most men.

The Institute is now in process of reorganisation as a central agricultural research station for the East African group of British Colonies and Protectorates, comprising Uganda, Kenya, Tanganyika, Zanzibar, Nyasaland and Northern Rhodesia. This represents a vast stretch of country reaching from the Zambesi to the sources of the Nile, within which agriculture is practised at altitudes ranging from sea-level to near 10,000 ft. Whatever Amani may lack it will not be subjects for investigation.

The establishment and functions of the proposed chain of research stations in the tropical and sub-tropical colonies have formed a principal subject of discussion at the recent Imperial Agricultural Research Conference. Opinion is unanimous that

there should be no interference with the full development of the territorial departments of agriculture. The work of the central station has been variously described as "fundamental," "long range," and "wide range" research, terms which it is probable that no two of us would define alike, but from which the general idea can be recognised. The officers working in agricultural departments have usually so many duties and problems pressing for attention that even the specialist in any particular branch has usually to be content if he arrives at a working solution, however empirical it may be. It will be the duty of the research station to seek the underlying principles from which the practice most suitable to any given set of conditions may be derived.

The broadest and most fundamental type of investigation lies in the study of soil and climate, and of the results of their interaction as expressed in the flora and fauna, both natural and artificial. An effective soil survey must be preceded and accompanied by a great deal of work on the nature and classification of East African soils. While this proceeds valuable information may be obtained by a shorter route through ecological studies.

There is another general group of what may be termed co-ordination services which the Institute may appropriately undertake. Internally these include comparative studies of the distribution and adaptation of crop-plant varieties, and of the distribution and incidence of pests and diseases. Externally they include plant introduction and plant protection against the admission of pests and diseases from other countries.

Of the innumerable subjects for special investigation which offer themselves only one or two outstanding examples can be given. The conservation of soil fertility will some day become a subject of deep concern to the European settler. At present he is living on the accumulated reserves of virgin soil. Wastage under cultivation is rapid, and wonderfully rich as the soil may be, and often is, it will not last for ever. The most general native system of regeneration is based on shifting cultivation, usually with a weed or bush fallow. It is worth study to find exactly how its effects are produced, the degree of its efficiency, and its capabilities for improvement.

The problems I should select as of most urgent importance are concerned with the behaviour under East African conditions of the Arabian coffee plant. In many areas the permanence of the plantations is open to serious doubt unless appropriate methods of handling the cultivation are worked out. The trouble known as overbearing is often serious, in which young trees suffer from serious dieback associated with what is regarded as prematurely heavy cropping. There are also peculiar interferences with normal growth attributed to extreme fluctuations of temperature. The most direct influence which can be used to control the conditions giving rise to these and other troubles is through the provision of shade, but on this subject there is confusion of counsel and the utmost diversity of practice. The establishment of principles in these matters presents primarily a series of problems in plant physiology, but will need to be approached through the combined efforts of workers in all the main branches of agricultural science.

The suggestions which I have put forward as examples of the work which may be undertaken by the central research station are entirely my own, and must not be understood to commit any superior authority concerned. The subject is still an

open one, and one which may very appropriately be discussed in a meeting of economic biologists.

The paper was illustrated by lantern slides and maps showing the situation of the Research Institute and the field and laboratory facilities for investigation.

ORDINARY MEETING of the Association held at 2.15 p.m. on Friday, November 18th, 1927, in the Imperial College of Science. Vice-President, Dr A. D. IMMS, M.A., in the Chair.

SYMPOSIUM ON "FOOT AND MOUTH DISEASE."

I. "History. Foot and Mouth Disease in Farm Animals. Disinfection" by F. C. MINETT, Esq., D.Sc., M.R.C.V.S., Institute of Animal Pathology.

II. "Experimental Foot and Mouth Disease in Small Animals. General Characters of the Virus. Immunity" by A. ARKWRIGHT, Esq., M.D., F.R.S., The Lister Institute of Preventive Medicine.

III. "Physical Properties of the Virus, Filtration, etc. Prophylactic Vaccines" by S. P. BEDSON, Esq., M.D., Hale Laboratory, The London Hospital.

IV. "Survival of the Virus Outside the Body" by Mrs Y. M. BURBURY, M.A., The Lister Institute of Preventive Medicine.

V. "Demonstration of the Lesions of Foot and Mouth Disease in Guinea-Pigs" by I. A. GALLOWAY, Esq., B.Sc., M.R.C.V.S., National Institute of Medical Research.

I. FOOT AND MOUTH DISEASE IN FARM ANIMALS.

BY F. C. MINETT, D.Sc., M.R.C.V.S.
(*Institute of Animal Pathology, London, N.W.*)

I WISH to make it clear at the outset that I am not permitted to discuss the policy which has been adopted by the Ministry of Agriculture in Great Britian for suppressing this troublesome disease. Briefly this policy consists of the slaughter of affected animals and of healthy susceptible animals on the same premises with restriction of movement of susceptibles within a wide area of country.

One of the principal reasons, I assume, for our presence here to-day is to describe the results of some of the research work which has been carried on in this country during the past three or four years. In the twenty minutes at my disposal it will not be possible for me to refer at length to that part of the subject on which I am expected to speak. For more detailed account those interested should consult the various publications which have appeared on the subject.

Recent research in this country has been conducted with the co-operation of various workers, both medical and veterinary, on behalf of a Committee which was appointed by Mr Noel Buxton, a former Minister of Agriculture, in 1924. This Committee is still sitting. Its present Chairman is Sir Charles Martin, F.R.S., Director of the Lister Institute. So far two Progress Reports have been issued, the first in 1925 and the second in the early part of 1927.

Work is proceeding at the present time in the following institutions: at the Veterinary Laboratory of the Ministry of Agriculture at Weybridge under the direction of Dr W. H. Andrews; the experiments on large animals are conducted at an isolation station at Pirbright, Surrey; at the Lister Institute of Preventive Medicine under the direction of Dr J. A. Arkwright; at the National Institute for Medical Research under the direction of Capt. S. R. Douglas. I understand work is to proceed at the Public Health Laboratories in Manchester under Dr H. B. Maitland.

In order to make my further remarks intelligible it is now necessary to say something of the cause and nature of the disease and of the animals which it affects naturally.

Foot and mouth disease is caused by a virus belonging to the filterable class. The fact of its filterability was proved exactly thirty years ago by the German investigators, Loeffler and Frosch, and it is of interest to note that this was the first disease of animals shown to be due to a virus of this class. In nature it affects cattle, sheep and other ruminants, such as deer, and also swine. The goat is occasionally attacked, while it is important to note that the horse is immune. A good many reports are to be found in the literature of the disease in human beings, especially children, frequently contracted it is supposed by the drinking of milk containing the virus. Possibly many of the reports of the disease in man should be treated with a certain amount of scepticism. Among small experimental animals the guinea-pig can be infected with virus direct from cattle.

In natural cases in the ox after an incubation period which usually lasts three to five days, but which may extend to ten days, there is a sharp rise of temperature immediately followed by an eruption of vesicles on the mucous membrane of the tongue, gums, and/or on the dental pad. Vesicles frequently appear also around the coronary margins of the hoofs and occasionally in other places such as the skin of the teats. Owing to their situation many of these vesicles quickly lose their epithelial covering and the contained vesicle fluid or lymph is discharged. In the case of the mouth vesicles this is accompanied by considerable salivation and this is frequently the first symptom which draws attention to the disease. The rupture of the vesicle and loss of epithelium leads to the formation of a shallow ulcer which usually heals over in the course of a week or so.

When lesions of the feet are at all extensive the animals display evidence of great pain and lameness, shaking their feet and strongly objecting to being forced to move. Apart from salivation, which incidentally may not be noticeable in the sheep and pig, it is this suddenly appearing lameness, often affecting simultaneously animals of different species, which first attracts the attention of the stockowner. At times and especially in pigs the lesions on the feet are so severe that the hoofs are shed.

Economic importance.

The enormous economic importance of foot and mouth disease to the farming industry in various parts of the world does not lie in the fact that it causes a heavy mortality but to the fact of its general debilitating effect upon the animal and the serious interference with trade arising from its highly infectious character.

Animals which have passed through an attack often show great loss of condition, due in part to the pain of mastication causing refusal of food. Pregnant animals have been known to abort and in milking cows the secretion of milk is often noticeably

diminished. The disease is liable to be particularly severe in young stock (*e.g.* young calves) and in some outbreaks there is a considerable mortality. According to estimations carefully compiled by Rudovsky in Austria before the war, the average loss per head of cattle may be put down as 98 kronen.

The measures of control which have to be adopted by European governments inevitably lead to considerable interference with animal movement, *e.g.* stoppage of markets, and consequently are in themselves a source of great financial loss. Further, outbreaks from the point of view of this country are most unfortunate because they lead automatically to an embargo upon the exportation of valuable pedigree stock to countries overseas.

Distribution.

At one time or another the disease has been found in most parts of the world in which susceptible animals exist. One noteworthy character which foot and mouth disease shares with some other epidemic diseases is the irregularity of its occurrence from year to year. In European countries, such as Germany, France, Belgium, Holland, Denmark, the disease has caused enormous losses. In Germany, for instance, in 1892 it affected one and a half million cattle, over two million sheep and nearly half a million swine. In Holland in 1907 there were 341,000 cases in eleven provinces. In one province of Austria during the period 1910–12 over 233,000 animals were reported to be diseased and the loss entailed amounted to at least twenty million kronen.

Compared with continental countries the position of Great Britain is in many ways far more fortunate. The first outbreak of which there is authentic record occurred in 1839, and since that time there have been very extensive outbreaks in different parts of the country. For instance in 1871–2 the number of animals attacked was estimated at three millions and it was in connection with this outbreak that legislation was first introduced. There have, however, been several periods of years in which no outbreaks at all have occurred, *e.g.* 1886 to 1892, 1895 to 1899, 1903 to 1907. As recently as 1923 there were 1929 outbreaks spread over forty-three counties, in the course of which 69,000 cattle diseased or in contact were slaughtered, besides numerous sheep and pigs.

Infectivity.

As has been stated above foot and mouth disease is extremely infectious. There is reason to believe that the infectivity is very high in the early stages of the attack, at the time when the temperature is raised and even before vesicles can be seen on the mouth or feet.

The German investigators, Waldmann and Reppin, have shown recently that virus may be present in the saliva before vesicles appear in the mouth. Certain experiments of Nicolau and Galloway at the National Institute for Medical Research have indicated however that the virulence of the saliva may be due to the existence of microscopic lesions of the mucous membrane.

As the attack progresses the infectivity of the animal gradually diminishes. Waldmann and Reppin state that as soon as the mouth ulcers are covered with granulation tissue the saliva ceases to be infective. Lebailly has called attention to the fact that four days after the appearance of vesicles the ox may be no longer capable of transmitting the disease by contact.

Experimental production in farm animals.

Experimentally the disease may be set up in susceptible farm animals by inoculation of vesicle fluid or epithelium from cattle, sheep or swine. Continental observers have remarked upon the ease with which it is possible to infect large animals by rubbing the virus into scarified areas of the buccal epithelium such as of the tongue or dental pad. Judging by experience with the guinea-pig injection of the virus intradermally in the same regions should be a still more certain method. As with guinea-pigs too, larger amounts of virus are required to infect by the subcutaneous, intramuscular or intravenous routes. On a few occasions we have failed to set up clinical symptoms of the disease when virus was given intramuscularly, this being the route which has been adopted as a routine up to the present. When virus is given by this channel a rise of temperature to 105° F. or over may be expected within seven days and this is followed by the appearance of vesicles at the predilection sites. The vesicles vary considerably in number from case to case and on the tongue may attain the size of a five-shilling piece. There is some evidence from clinical observation that on the whole lesions are larger and more extensive in fully-grown animals than in immature ones.

According to Waldmann and some other observers the disease in the ox and pig resembles that in the guinea-pig in that when strong virus is scarified on to the mucous membrane of the lips or gums a local or primary lesion develops at the site of scarification, which is followed later by secondary lesions on the mouth or feet. We have had little experience of this method of infection though we have on a few occasions failed to note any clearly defined local lesions in large animals.

It has been said that the virus on being continually passed through the same species of animal such as the ox loses much of its virulence for this species and with the object of maintaining the virulence alternate passages through two different species such as the ox and pig have been recommended. So far no decided drop in virulence has been noted with cattle virus maintained in cattle, but as has been remarked by others strains of virus which are maintained in guinea-pigs lose in a considerable degree their power to infect cattle. The possibility was investigated a few years ago that this might prove to be a method whereby a permanently attenuated virus suitable for the immunisation of cattle could be obtained. Unfortunately this proved to be impossible since a virus adapted to guinea-pigs is still capable of infecting cattle and after a few passages in the larger animals appears to regain its normal power of infecting them.

In the work at Pirbright cattle have been largely relied upon as experimental animals. Swine have also been used fairly extensively though the lesions in these animals are liable at times to be limited in extent. Sheep are on the whole unsuitable since lesions in our experience are frequently insignificant or cannot be detected at all.

Different types of virus.

In 1922, as the result of experiments on the protection afforded by an attack of foot and mouth disease in cattle, two French investigators, Vallée and Carré, came to the conclusion that two types of virus existed in nature. These were designated *A* and *O* respectively and were stated to be quite distinct inasmuch as cattle solidly immunised against the one were still quite susceptible to the other. From the clinical point of view there was little to distinguish them.

It appeared to the British Committee to be very desirable that these findings should be investigated in this country. For this purpose samples of ox blood carrying the two viruses were received from M. Vallée in 1925 and were tested on cattle, sheep and swine. A certain number of tests were carried out at the same time on guinea-pigs. The main conclusions derived from this work were that for all practical purposes the viruses were distinct immunologically. Cattle immunised to one type by two consecutive injections were still susceptible to the other type when this was inoculated a month or so later. In fact there was clinical evidence to show that frequently the second attack was more severe than the first. The types, however, were not entirely distinct since in 23 per cent. of experiments there was sufficient cross-immunity to prevent the development of detectable lesions after the injection of the second type of virus.

The practical distinction between the two types was clearly demonstrated by three accidental infections which occurred at the experiment station. In these cases cattle which had been proved experimentally to be immune to one type contracted shortly afterwards by natural means a second infection and material from this second infection was proved to contain virus of the second type. In order to gain information as to the distribution of the two virus types in Great Britain, seventeen strains from twelve separate centres of disease were tested on cattle, sheep, swine and guinea-pigs. The results indicated that of the seventeen strains examined one alone conformed to the *A* type, the others being indistinguishable from the Vallée *O* type.

Since these experiments were carried out the matter has been carried a step further by Waldmann and Trautwein, who report that they have been able to distinguish three main types of virus. I understand this has largely been confirmed at the Lister Institute by experiments on guinea-pigs.

Epidemiology.

It is not possible in the time at my disposal to enter into this subject in detail or to refer to the numerous methods by which the disease may be spread. I wish merely to recite a few facts regarding the period of survival of the virus within the tissues of animal carcases.

It is recalled that in 1926, as the result of inoculation experiments at Pirbright, irrefutable evidence was furnished the Ministry of Agriculture that in the particular instances in question foot and mouth disease had been imported into this country from the Continent through the medium of fresh pig carcases. Owing to a certain infected consignment the disease spread to forty-five separate farms, involving the slaughter of 2626 animals at a cost of nearly £41,000 in addition to disinfection costs. As the result of these findings it was decided to investigate some of the conditions of survival of the virus in the tissues of animal carcases, particularly in animals killed before the period of active vesiculation.

A series of experiments was conducted in the first place with guinea-pigs. These animals were killed at the time of blood infectivity by stunning and immediate bleeding and their carcases dressed in butcher's fashion. The results indicated, as might have been anticipated, the predominating influence of temperature on the survival of virus. At temperatures of 2° C. to 7° C. the virus remained alive in the blood for 21 days and in the bone marrow up to 87 days and in the lesions themselves for at least 102 days.

Experiments of a similar nature were then carried out with the carcases of large animals. Cattle and swine were infected by intramuscular inoculation, killed by a butcher at the time when the blood was infective and the carcases dressed in trade fashion. The carcases were subsequently stored at trade freezing (10–15° F.) and chilling (28–30° F.) temperatures and at intervals material was removed for inoculation of cattle and swine and for the feeding of swine. A few bacon carcases were also treated by wet-salting and by dry-salting processes such as are used in the trade. The main results were as follows:

There was no evidence to show that the muscular tissue retains a dangerous degree of infectivity either by inoculation or feeding. On the other hand, the bone marrow was capable of setting up infection for at least forty-two days in the case of bacon carcases stored at freezing or chilling temperatures or treated by wet or dry-salting processes. In the case of both a beef carcase and a bacon carcase stored at freezing temperature the bone marrow contained virus after seventy-six days. A further point of great practical importance was that the disease may be transmitted to swine fairly readily by feeding them upon crushed bones containing infective bone marrow. On five occasions out of seven such feeding gave positive results and was presumably due to small injuries produced by spicules of bone since the feeding of marrow alone failed to convey the disease. These experiments suggest certain rather obvious possibilities from the point of view of the meat trade of this country.

Disinfection.

I can only summarise briefly the work which has been done on this subject and which has occupied a good many months.

A study has been made of the resistance of the virus to a number of chemical reagents, some of which are in common use as disinfectants. The virus used has been put up in two forms, viz. vesicle fluid from the primary lesions of guinea-pigs diluted with saline and filtered, and as small discs of infective guinea-pig epithelium. The discs of epithelium for the purposes of experiment were suspended in a medium containing ox faeces and ox saliva to provide additional organic matter.

Trials were made by mixing the virus-containing medium with the reagent dissolved in water (where this was possible) to double the required concentration. Exposures were at laboratory temperature for three hours in the case of filtered vesicle fluid and for twenty-four hours in the case of the faeces medium, after which the material was tested for survival on guinea-pigs.

From a practical point of view the most important result arising from this investigation was the relative inefficiency of phenol and cresol and of certain coal-tar disinfectants. In the case of filtered virus in which the proportion of organic matter is low, the salts of certain metals, *e.g.* copper sulphate, zinc chloride and mercuric chloride are far more destructive than phenol. For instance, under the conditions of the experiments a concentration of 2 per cent. phenol is required to destroy the virus in filtrates, while copper sulphate is usually active at a final dilution of 1 in 10,000 and zinc chloride at 1 in 1000.

From the point of view of practical disinfection, however, no more efficient reagent has been found than formalin. The infectivity of guinea-pig epithelium in the faeces medium is destroyed by formalin in a final concentration of 1 in 600 with an exposure

of twenty-four hours at room temperature. Formalin can be tolerated by the operator when used for spraying the insides of buildings at a concentration of 1 in 100, though other methods such as fumigation might be more suitable.

The reagent has also been shown to be of great value for the disinfection of animal fodder such as the outsides of ricks and for the disinfection of animal hides.

II. EXPERIMENTAL FOOT AND MOUTH DISEASE IN SMALL ANIMALS.

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Modern knowledge of foot and mouth disease may be said to have begun with the work of Loeffler and Frosch (1) whose report was published in 1897–8. Their experiments were conducted almost exclusively on farm animals, since they failed to infect experimentally any animals smaller than swine. Their results constituted a great advance on previous knowledge. For instance, they showed the very high concentration of the virus in vesicles on the calf and pig. They also established the filtrability of the virus and its slight resistance to heat, which is less than that of ordinary bacteria, and found that it was comparatively rather resistant to carbolic acid (0·5 and 1·0 per cent.) and to some other disinfectant substances. Loeffler and Frosch also showed that an "anti-serum" could be obtained from cattle which had recovered from the disease. This serum had neutralising properties for the virus *in vitro* and could also be used for giving passive immunity to cattle by inoculating them with rather large doses. For this purpose they used the serum of animals which had passed through the disease and had also been hyperimmunised by inoculating them with further doses of living virus. Loeffler and Frosch could not demonstrate any immunising effect following the injection of virus which had been killed, or acted on by heat or chemical substances in such a way as to destroy its infectivity.

Experiments on Animals.

Since animal experiments were essential for detecting the presence or activity of the virus, they were very much hampered by the necessity of using large animals, such as young pigs and calves. The consequence of this handicap was that they experienced great difficulty in establishing the best methods of preserving the virus, in deciding on the best means of procuring virus and finding out whether any given sample was active or not, and if so, how great was its potency. Since every test had to be carried out on a series of at least from three to six swine, it is not surprising that efforts to standardise an extremely variable virus, or the anti-serum, could not be carried out with great accuracy nor as often as was desirable. In the recent work at the Lister Institute, it has been found desirable to estimate the titre of every sample of virus used for experiments, by the use of five or six animals, and all subsequent experiments, of course, require further batches of varying numbers.

Attempts by Loeffler and Frosch to induce a very mild but protective attack of the disease in cattle by giving small doses of virus together with immune serum, gave irregular results, sometimes inducing too severe an attack and sometimes

failing to produce immunity, with disastrous results later. There can be no doubt, then, that this unsatisfactory state of things was in large part due to the difficulty in standardising materials and establishing uniform methods by a sufficiency of experiments.

Susceptibility of the Guinea-pig.

It is at first sight remarkable that the discovery of the susceptibility of the guinea-pig was so long delayed.

Waldmann and Pape (2) announced their technique for the successful and regular infection of guinea-pigs in 1920, and published a full and convincing account in July 1921. The failure of others is partly to be attributed to the difference in the virulence for guinea-pigs of different strains of virus. Frequently there is need for passage through several guinea-pigs before a well-developed lesion can be obtained in these animals with virus from the cow. The different strains of virus also vary in their adaptability to the guinea-pig. Guinea-pigs of 350 gm. or more are found to react with larger and better developed vesicles than do smaller animals.

Method of Inoculation.

The best way of infecting a guinea-pig is to inoculate virus intradermally in the sole of the hind foot. This is done by making three or four longitudinal tunnels with a hypodermic needle in the thickened skin of the plantar surface and leaving a little fluid in each needle track. The needle in this technique passes through the mass of the Malpighian layer of the epidermis and just taps the extremities of the dermal papillae causing slight haemorrhage. Inoculation may also be made very successfully by scratching the plantar skin and rubbing in the virus. If an active and well-adapted virus has been used, 24 hours later vesicles will have begun to form along the needle tracks and by about the 36th to the 48th hour the whole of the skin of the sole is involved in one large vesicle. It is at this period that the vesicle fluid affords the most potent virus.

Source of Virus.

A few drops only can be obtained from one guinea-pig but this diluted 100,000 or 1,000,000 times, or often 5,000,000 or 10,000,000 times, is still virulent and in a state to reproduce the same effect in another guinea-pig.

Further, this vesicle fluid diluted 1 in 50 with slightly alkaline salt solution and filtered through a Chamberland, Berkefeldt or Seitz filter may be kept in the cold room with little loss of activity for weeks or months. Some samples, if kept at a pH of 7·6 remain highly infective for considerably over 12 months.

At the early stage of the disease, when the vesicle fluid is at its maximum potency, the epithelium covering the vesicles is also very highly virulent and if cut off and placed in equal parts of glycerine and salt solution in the cold it will remain very potent for months or years.

Such vesicle epithelium, if removed from the animal and dried in the air, has been recently shown by Trautwein (3) to be still active after some weeks.

Course of the Disease.

If the course of the disease in guinea-pigs is traced, there is found to be a close similarity to the experimental infections of cattle, sheep and swine.

As has been seen, primary vesicles appear at the site of inoculation on the plantar pad at about the 24th hour and at the 48th to the 72nd hour secondary vesicles

appear on the uninoculated feet and in the mouth, on the tongue and lips and gums. The tongue is especially affected and, if the lesions here are severe and on the posterior section of the tongue, they are accompanied by profuse salivation. There may also be diarrhoea. A small percentage of animals, sometimes 5 per cent., die, wasted, in 10 to 14 days. In animals which recover, the healing is as a rule very rapid.

Distribution of Virus in the Body.

In studying the course of the disease, it is important to observe where, in the body, the virus is most concentrated. Nowhere does the potency of the virus equal that in the primary vesicle, *e.g.* a titre of 2,000,000 to 5,000,000, though it may reach 50,000 or 100,000 in the secondary vesicles. The size and development of the secondary lesions, moreover, are proportional to a large extent to the size of the primary lesion. If, at the site of the intradermal inoculation, no vesicle or only a very small one, occurs, on account of insufficient dosage, etc., the secondary lesions are absent.

At the time when the primary vesicles are well developed, the blood and internal organs also contain virus, but the concentration here is relatively low, about 1 in 5000 dilution of blood is infective, *i.e.* there is about 1000th of the concentration in blood that there is in vesicle fluid. In the internal organs the concentration is no higher than in the blood.

If the plasma or serum and corpuscles are examined separately, the bulk of the virus is found in the liquid fraction. Plasma contains about seven-eighths of the total virus in a sample of blood.

Site of Propagation.

These facts, as to the virus being so much more concentrated in the vesicles than elsewhere, point to the virus being propagated for the most part in special parts of the skin and buccal mucous membranes. Whether the virus increases chiefly or solely in the epithelium rather than in the sub-epithelial connective tissue it is not so easy to say for certain, though microscope sections suggest this. It has generally been accepted that the epithelium is the chief seat of propagation, on account of the macroscopic and microscopic appearances, but other kinds of damage done to the dermis may result in vesicles, as happens in a burn, and as Ledingham (4) has shown reason to believe occurs in vaccinia. Virus is present in high concentration in the saliva and mouth discharges at the height of the disease, but this is probably chiefly derived from the vesicles in the mouth, though it has been detected in the saliva from Steno's duct and has also been found in the milk and more rarely in the urine, but not in the faeces.

Decline of Infectivity.

A very important observation was made by Lebailly (5) and by Vallée and Carré (6) to the effect that the infectivity of cattle rapidly decreases after the appearance of vesicles. It had been generally assumed that all the characteristic and copious mouth discharges were highly infective at all stages, or at any rate, that whether in any given case they were infective or not was a matter of uncertainty.

In guinea-pigs this fact is represented by the very rapid decline of the activity of the blood, which begins at the time that the secondary vesicles appear, and of the contents of all the vesicles after the third or fourth day from inoculation.

This disappearance of virus must be due to active destruction in the body and in the skin and epithelium so long as it is attached to the body, since, on the other hand, epithelium which has been removed at the time of its greatest virulence remains very active for months if suitably kept apart from the body. The decrease in activity of the epithelium whilst attached to the rest of the skin cannot, therefore, be due to simple cessation of growth and spontaneous decay.

Course after Inoculation on Hairy Skin and into Muscles.

If guinea-pigs are inoculated in other positions in the body than the soles of the feet or buccal mucous membrane, the dose necessary for infection is a much larger one, *e.g.* 100 to 1000 times. Nevertheless, guinea-pigs can be regularly infected by injection intramuscularly or subcutaneously with a sufficiently large dose of virus. Intradermal inoculation on the hairy skin is a very uncertain procedure, for a local vesicle does not form and as a rule, when local reaction fails after a small dose, the virus is not propagated. Any resulting infection must then result from escape of the inoculated virus from the wound into the blood and its chance arrival at a suitable site in the skin in sufficient quantity to set up a local lesion and accompanying multiplication of virus.

In the same way when the inoculation is intramuscular, no local reaction is visible to the naked eye and very little microscopically, and probably little or no local multiplication occurs, but after 24 to 48 hours, vesicles appear on the feet and in the mouth. These behave like secondary vesicles, they may be quite large and contain highly virulent fluid.

Immunity.

Immunity follows any attack of foot and mouth disease in the guinea-pig which is accompanied by well-formed vesicles. It is recognisable *at least* as early as the 7th or 10th day after inoculation and lasts four to six or even 12 months.

The immunity when at its height is shown by absence of reaction to any inoculation, even when given intradermally. After three or four months this high grade of immunity wears off and the animal reacts with local lesions when inoculated intradermally with a moderate dose of virus, but no secondary vesicles follow. At this time no attack results from intramuscular inoculation, even of a large dose. Later still, primary and secondary lesions may follow intradermal inoculation but a residual immunity is shown by the inefficacy of intramuscular inoculation to produce the disease. Later still, when the resistance has completely disappeared, even intramuscular inoculations are successful.

Antibodies in the Serum.

At the same time that the resistance to inoculation appears it is possible to detect changes in the serum, so that this is able to neutralise virus *in vitro*; also when the serum of an immune animal is inoculated into a normal susceptible animal, this too becomes resistant. Such "passive" immunity is due to "antibodies" in the blood, analogous to those arising as the result of bacterial infections. The titre of such antibodies in the serum is never very high but 0·4 c.c. may give protection to a guinea-pig of 300 gm. against several thousand infective doses (Waldmann states that 20 c.c. of hyperimmune serum per cwt. is necessary to give good protection to cattle).

The protection thus afforded to guinea-pigs is not complete. When a passively immune animal is inoculated intradermally on the foot a local vesicle always follows but no secondary vesicles. The intradermal inoculum appears to be able to resist or avoid the circulating antibodies in the blood. Intramuscular inoculation is unsuccessful during this period.

This passive immunity which protects guinea-pigs from generalised lesions is interesting since it is presumably parallel to the state of protection afforded to cattle by serum. The cattle appear often to be completely protected against natural infection for about 10–14 days, but it is a question whether they really entirely resist the primary local infection or whether this is merely prevented from developing and extending; and whether they would be immune to intradermal inoculation.

Types of Virus.

The immunity afforded to guinea-pigs, as to cattle, by one type of virus is little or no protection against other types. As cattle may have three attacks within a few months, produced by experimental or natural infection with the three types in succession, so guinea-pigs may be infected with the three different types, Waldmann *A*, *B* and *C* within a few weeks. The fact that differences of type are as readily distinguished in guinea-pigs as in cattle has enabled experimental work to clarify our knowledge of the distinct types and to ascertain the type present in a given case with comparative ease. This may be done by immunising different guinea-pigs separately with the three different types, either by giving them an attack of the disease, or by the quicker and easier process of vaccinating them, or more rapidly still, by passively immunising them with serum of the several types, and in any case subsequently testing them for immunity to the unknown virus.

Natural Infection in the Guinea-pig.

Though the disease in guinea-pigs is so regular and well-marked after inoculation, cases of direct spontaneous infection from one to another are rare, even in animals in the same cage unless intentional wounds and contamination of the litter are provided. Also guinea-pigs do not readily contract the disease from cattle.

Foot and Mouth Disease in other Small Animals.

Something remains to be said about foot and mouth disease in small animals since it might be very important practically if foot and mouth disease could be shown to be an easily acquired and communicable disease in indigenous small animals.

It may be said at once that none of those animals investigated show any high degree of susceptibility, that even direct inoculation is much less successful in them than in guinea-pigs, and that as in guinea-pigs, natural infection from one to another has hardly ever been observed. Several species can, however, be given foot and mouth disease by inoculation.

Rabbits can be infected by inoculation of the tongue and the vesicle formed there may yield virus which is highly infective for guinea-pigs, but the course of the disease is very short and irregular and the vesicles heal very rapidly. Rabbits only have lesions in the mouth where secondary lesions also appear. The blood is infective for two days and otherwise they behave in a similar way to guinea-pigs. The disease can be passed by inoculation from rabbit to rabbit. In two cases the disease was

passed spontaneously from one rabbit to another by wounds made in fighting, otherwise no natural infection has been observed.

Wild rats can be infected with virus from guinea-pigs by simultaneous inoculations, intramuscular and intradermal, on the foot. Vesicles appear on the feet and tongue and may be to some extent adapted to the rat by passage, and intradermal inoculation alone of the adapted virus will produce the disease. No transmission of the disease was effected by contact or by feeding with infected epithelium.

Tame or white rats were still less susceptible though local vesicles could be produced by inoculating the tongue and passing in the same way to other white rats, no adaptation was obtained nor was there any generalisation. Fifteen white, or tame, mice were inoculated unsuccessfully; neither vesicles nor infectivity of the blood resulted. Ten house mice were inoculated intramuscularly, but no vesicles appeared. The blood, however, was infective on the 2nd day in some instances.

Twelve wood-mice (*Apodemus sylvaticus*) inoculated intramuscularly, showed slight evidence of infection, occasionally very small vesicles appeared on the tongue and the blood was regularly infective for guinea-pigs on the 2nd and sometimes on the 5th day. Wood-mice did not contract the disease naturally from their companions.

A *hedge-hog* was also infected by inoculation by F. C. Minett.

Birds. The blood of fowls, after inoculation, may be infective for guinea-pigs, but lesions have not been observed and the excreta of birds that had been fed with large quantities of infective epithelium very rarely contained active virus. Farmyard fowls, sparrows, and martins, were used in these experiments.

Cats and dogs can be infected only with difficulty, the lesions are inconsiderable. The inoculated disease is, however, apparently often fatal to kittens and puppies but the lesions are slight and spontaneous infection was not observed. Though Loeffler records that dogs were found on an infected farm with mouth vesicles, his attempts at inoculation were unsuccessful.

General Characters of the Virus.

The particles of which foot and mouth virus consists must be extremely small on account of their very ready filtrability. Olitsky and Boëz (7) considered that they were positively charged at a *p*H more acid than about 8·0, but difficulties arise in examining the charge on particles in albuminous fluids at *p*H in these regions.

The virus is very sensitive to certain disinfectants and very resistant as compared with bacteria in others, *e.g.* alcohol, chloroform and glycerine.

For long it was believed that it was especially readily destroyed by drying and Loeffler in 1909 had come to the conclusion that any survival of the virus outside the body must be in a moist condition. Nocard, Roux, Vallée and Carré (8) showed that this was not universally true. Recent work by Y. M. Burbury has discovered very remarkable facts in this connection.

What is now known about the survival of the virus on inanimate objects may have a very important bearing on the spread of the disease to farm animals, but the application of the knowledge gained to practical administration must await further experiments in which the manner of the infection of the larger animals themselves is investigated.

Cultivation.

No method of cultivating the virus in artificial media has yet been discovered and the claims that this has been done have not stood the test of repetition by the originators and others. Very many methods have been attempted in media of very varied composition under aerobic and anaerobic conditions.

Blood serum has been a component of practically all the media used by those who have claimed success in culture. Bedson and Maitland, however, found that *in vitro* serum diluted 1 in 10 or stronger was definitely harmful to the virus, but that this noxious property was removed or lessened by adding a piece of sterile animal tissue or of raw potato to the liquid. They believed that this difference was connected with changes taking place at the surface of the pieces of tissue.

The virus is generally assumed to be a living micro-organism, but the problem of the nature of filtrable viruses which apparently can only multiply when in contact with other living things, is largely a matter for speculation. The problem of foot and mouth virus meets much the same difficulties as those connected with the viruses of vaccinia, of Rous sarcoma and the Bacteriophage. The problem can scarcely be said to have been solved yet by direct experiment or observation.

The solution seems at present to be determined in the minds of writers on the subject by analogies which are not very close and by preconceptions derived from general views on the nature of living things and the properties by which it is supposed that they can be definitely separated from the not-living.

For further details of the investigations on foot and mouth disease in this country and for a bibliography of the recent literature, the reader is referred to the Progress Reports of the Foot and Mouth Research Committee of the Ministry of Agriculture. Two of these reports have already been published, 1925 and 1927, by H.M. Stationery Office, London.

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III. PHYSICAL PROPERTIES OF THE FOOT AND MOUTH VIRUS.

By S. P. Bedson, M.D.
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Physical properties of the virus. A knowledge of the physical properties of the virus of foot and mouth disease is of importance, not only because of its practical application in the study of the virus, but also for the light which it may throw on the nature of the virus. The nature of filtrable viruses has been made the subject of much investigation and speculation, but the question is still a long way from solution, and of the various hypotheses put forward the two most worthy of consideration are:

(1) that these viruses are organised particulate living things which differ only from the known cultivable bacteria in being smaller and more highly specialised parasites;

(2) that they are enzyme-like, though differing from the enzymes so far studied by the biochemist in that they are capable of increasing in quantity when acting on their specific substrates—the living tissue cells.

Although the knowledge gained of the properties of the foot and mouth virus does not enable us to say whether or not it is a living thing, some interesting observations have been made which should be of assistance in providing the ultimate solution to this riddle.

Filtrability. The filtrability of this virus, first demonstrated by Loeffler and Frosch in 1898, has been amply confirmed by subsequent work and it is now known that the foot and mouth virus passes through Berkefeld *N*, Chamberland L_1*, L_2 and L_3, Mandler (6–12 lbs.) and Seitz filters with comparatively little loss (Bedson and Maitland(1), Minett(4)). Olitsky and Boëz(5) have confirmed this work and have shown in addition that at a pH of 7·5 the virus is capable of traversing Chamberland L_7 and L_9 filters but not L_{11}. They have, however, produced evidence which suggests that the foot and mouth virus carries a positive electrical charge at pH 7·5, the iso-electric point being in the neighbourhood of pH 8·0. This observation is based primarily on cataphoresis experiments, but finds support in the results obtained by filtration at different hydrogen-ion concentrations. It is known that the various filters used in this work (Chamberland, Berkefeld, Mandler, Seitz) are negatively charged. At pH 7·5 when the virus would be carrying an opposite charge to the filter it passes with difficulty through the Chamberland L_7 and L_9 filters, but not at all through the L_{11}. If, however, the filtration is carried out at pH 8·5 then it passes through all three. The size of the pores of the Chamberland L_{11} candle have been estimated by Bechhold to be 150 $\mu\mu$.

Ultrafiltration. Levaditi, Nicolau and Galloway(3) carried out some ultrafiltration experiments with this virus and found that it was capable of traversing 5 per cent. collodion sacs, which, though permeable to peptone and amino-acids, allowed the passage of only a trace of protein (1 part in 1000). Olitsky and Boëz(5), although unable to confirm this observation, have obtained evidence which suggests that the foot and mouth virus is particulate, though of extremely minute dimensions. They made use of discs of filter paper impregnated with varying concentrations of acetic collodion. Three per cent. membranes which allowed blue litmus and 1 per cent. Hb to pass through readily, only let through collargol and the virus occasionally (1 in 4 filtrations), whereas 1·5 per cent. membranes allowed collargol and virus to go through every time. These latter membranes held back colloidal arsenic trisulphide and since the size of the particles of this substance in colloidal state has been computed to be 100 $\mu\mu$ and that of collargol particles 20 $\mu\mu$, then the foot and mouth virus particles would be between 20 $\mu\mu$ and 100 $\mu\mu$ in size.

Centrifugation. The above observations find confirmation in the inability to concentrate this virus by means of the centrifuge. Bedson and Maitland(1) have shown that when foot and mouth virus in the form of vesicle fluid diluted 1 in 50 in saline is centrifuged for $1\frac{1}{2}$–2 hours at 5500 r.p.m. there is no evidence of any diminution in the virus content of the upper layer of the column of fluid centrifuged, nor is there any increase of virus in the lower layers. Olitsky and Boëz(5) have repeated these experiments and obtained similar results.

Prophylactic vaccines. Although it has been known for some time that recovery from an attack of foot and mouth disease is associated with the development of immunity, it has been held that no appreciable degree of resistance to the virus could be evoked without the production of lesions. The experimental work on guinea-pigs carried out by the Foot and Mouth Disease Research Committee has shown that this view is incorrect. Although virus killed by heat (55° C. 20–45 minutes) and by phenol was found to be useless as an immunising agent, when the virus was killed by means of formalin it possessed considerable immunising properties (Bedson, Maitland and Burbury (2)). This vaccine was prepared by adding 0·1 per cent. formalin to vesicle fluid diluted 1 in 50 with $M/50$ phosphate solution pH 7·6 and allowing the formalin to act for 48 hours at 26° C. The vaccine was then stored at 5° C. The following table shows that formalised virus is capable of conferring immunity on the guinea-pig and further that this immunity is rapidly developed.

Table I.

Guinea-pig	Immunising dose	Interval between immunisation and test inoculation	Test inoculation	Result
991	0·5 c.c. 1/50 formalised vesicle fluid inoculated intra-musc.	2 days	0·3 c.c. 1/100 vesicle fluid inoculated intra-musc.	Not protected
992		,,		Immune
993		,,		Not protected
994		,,		Immune
995		4 days		,,
996		,,		,,
997		,,		,,
998		,,		,,
999		5 days		,,
1000		,,		,,
2.1		,,		,,
2.2		,,		,,

The test dose employed (0·3 c.c. i.m.) regularly infected the control animals, but it was found that inoculation of the virus in the plantar skin was a more stringent test of immunity. When tested in this way a single dose of 0·5 c.c. formalised virus was found to give only a partial protection, local lesions developed at the site of inoculation but these were not followed by generalisation. This is, of course, a very severe test, but even so it has been found possible by means of formalised virus to protect guinea-pigs against 10 m.i.d. of virus introduced intradermally (plantar skin). Thus, of 7 guinea-pigs which had been immunised by 3 weekly doses of 1·0 c.c. formalised virus subcutaneously, 2 out of 4 resisted completely an intradermal test of 10 m.i.d. whilst the remaining 3 given 100 m.i.d. all developed local lesions. With regard to the size of dose of formalised virus required to produce immunity in guinea-pigs the following figures are of interest (Table II). These animals were all given one intra-muscular inoculation of the vaccine and were tested 6–8 days later by intradermal inoculation (plantar skin), the occurrence of immunity being determined by the absence of generalised lesions.

When the test was made by the intra-muscular route, a single dose of 0·002 c.c. vaccine was found to protect 3 out of 4 guinea-pigs against a dose of 0·5 c.c. 1 in 50 vesicle fluid, thus illustrating the greater severity of the intradermal test. Finally it has been found that the immunity produced by this vaccine lasts for at least

Table II.

Dose of vaccine c.c.	Number of guinea-pigs	Results
1·0	2	2 had no generalised lesions
0·5	18	15 ,, ,,
0·1	29	15 ,, ,,
0·01	16	All developed generalised lesions
0·002	4	,, ,,

95 days and that the vaccine retains its immunising power for a considerable time when stored in the cold. About the same time that this work was being carried out for the Foot and Mouth Research Committee, Vallée, Carré and Rinjard (6) published a short series of experiments on cattle in which they demonstrated that immunity could be produced in this animal by the inoculation of formalised emulsion of vesicle epithelium, and this was followed in 1926 (7) by a communication giving the results of further experiments demonstrating the efficacy of formalised virus as an immunising agent in cattle.

Quite apart, however, from the difficulty of obtaining sufficient virus to enable the preparation of the vaccine on a large scale, more extended experiments on cattle would be required before attempting to evaluate the employment of a formalised vaccine as a prophylactic measure for the control of foot and mouth disease in the field. The results obtained in guinea-pigs and the small number of experiments in cattle carried out by the French investigators are encouraging; nothing more definite than that can be said at the present moment.

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IV. SURVIVAL OF THE VIRUS OUTSIDE THE BODY.

By Y. M. Burbury, M.A.

(*Lister Institute, London, S.W. 1.*)

1. *Acidity and alkalinity, and their influence on the survival of the virus.*

It has been found (1) that the virus is very sensitive to the reaction of the medium in which it is suspended. The optimum reaction for the survival of the virus *in vitro*, at 37° C., was determined by putting up vesicle fluid in molecular 50 buffer phosphate solutions, at hydrogen-ion concentrations ranging from 6·2 to 8·5. The particular phosphate solution which enabled the virus to survive for the longest period (as tested on guinea-pigs), was one which maintained a hydrogen-ion concentration of 7·6. This has, therefore, been taken as the optimum reaction. This optimum

reaction for survival of the virus has been found to apply to all temperatures at which the virus is allowed to remain. For instance, virus kept in the cold room, at a hydrogen-ion concentration of 6·2, did not survive 69 hours. The same virus, at 7·6, was still alive at the end of a year. Thus, comparatively small deviations from the optimum reaction lead to rapid destruction of the virus. The following table shows the effect on the survival of the virus of the hydrogen-ion concentration of the diluting fluid.

Table I.

	Reaction *p*H					
At 37° C. for	6·2	7·0	7·4	7·6	7·8	8·5
22 hours	–	–	+	+	+	+
44 ,,	–	–	+	+	+	+
70 ,,	–	–	+	+	+	–
4 days	.	.	–	+	–	.
5 ,,	.	.	–	–	–	.

+ = Solution still infective. – = Solution not infective.

The same quantity of virus was introduced into each of the 6 buffer phosphate solutions; that is to say, 10,000 infecting doses. It is clear that 7·6 is the optimum reaction: at this *p*H it took 5 days' incubation to reduce 10,000 infecting doses to none. This reaction is roughly that of normal blood at 37° C. It is also the optimum for many common bacteria.

These facts show the importance of correctly adjusting the reaction of the medium in which the virus is suspended, as a starting point for further investigation of its properties.

2. *The effect of temperature on the virus.*

The virus is heat-sensitive, and, like most bacteria, is quickly destroyed by high temperatures. It has been found that the temperature best suited to its preservation is that of the cold-room. For instance, the virus in vesicle fluid was virulent after 190 days at + 4° to + 7° C.; and emulsions from pads of infective guinea-pigs, were virulent after 124 days at – 5° to + 3° C., in spite of successive freezing and thawing (2).

When the temperature was increased, the death-rate of the virus increased. Thus, at laboratory temperature a 1 in 50 dilution of virus in phosphate solution remained infective for at least 3 weeks.

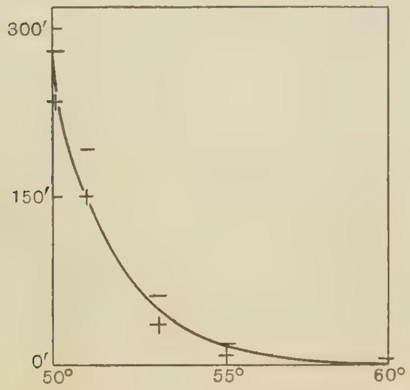
At 37° C. virus in this dilution has not usually survived for 1 week.

At 55° C. it died in 15 to 45 minutes; and at 60° C. it was killed within 5 minutes (3). As might be expected, therefore, the virus is instantly destroyed at the temperature of boiling water.

The sensitiveness of the virus to changes in temperature may be illustrated by means of a curve (Table II) showing the effect on the virus in infective blood of temperatures between 50° and 60° C. The blood was sealed in ampoules, and heated in water-baths for various periods.

It is seen from the curve that while the blood was still infective after 240 minutes' heating at 50° C., it was not infective at the end of 2 minutes heating at 60° C. (3). These results are comparable to those obtained by Matte and Sanz (4) on the effect of heat upon the survival of virus in infective ox blood.

Table II.



+ = Blood still infective. − = Blood not infective.

3. *The effect on the virus of drying, complete and partial.*

Observations on the spread of the disease in the field have frequently supported the belief that the virus may survive on inanimate objects for considerable periods. As the records of previous investigators on the survival of dried virus are somewhat contradictory, the effect of drying, under various conditions, has been investigated anew.

The virus was dried on glass slides, because these represent a common, inert substance. Virus, in the form of vesicle fluid, or infective blood, has been dried in two ways, either rapidly, in an air-current at 37° C., or slowly, at room temperature, in the air of the laboratory. It was found that slow drying was much less destructive than rapid drying. Subsequently, the dried virus has been kept in air at different water-vapour pressures, and at temperatures of either 18° C. or 37° C. By this means, the virus has been kept either chemically dry, over pure H_2SO_4, or else in an atmosphere 70 per cent. saturated, which represents roughly the degree of moisture contained in the air, on a warm, dry day, in this country.

The results of experiments such as these have shown that the virus, when dried on glass, survives for a long time, *i.e.* more than 2 years, at room temperature, if it is kept *chemically* dry. Yet it dies in about a week, if it is left in air under normal atmospheric conditions. Table III gives an example of the effect on the survival of the virus of complete, and partial, drying on glass-slides.

Table III.

Environment	Maximum survival
Chemically dry air 37° C.	7 days
„ 18° C.	>2 years
70 % saturated air 18° C.	5 days

It may be noted that even when chemically dry, the virus is rapidly destroyed at a temperature of 37° C.

4. *The effect on the dried virus of the substrate on which it is dried.*

Virus has been allowed to remain on a number of common fabrics and food stuffs, under ordinary atmospheric conditions, in order to discover whether any of these materials markedly prolonged its survival. (It will be remembered that on glass slides, at room temperature, and average saturation of the atmosphere, virus quickly died.)

Pieces of fabric, or small quantities of food-stuffs, were contaminated with the same quantity of virus, and were allowed to dry slowly, in air shielded from light. Subsequently, they were kept open to the air of the laboratory, either in a dark cupboard, or exposed to indirect sunlight. The average temperature, during this time, was 62° F., and the average degree of saturation of the atmosphere, 52 per cent. At intervals, samples of the material were tested for infectivity by inoculation of their contents into guinea-pigs.

It was found that on fabrics such as paper, silk or wool, on sand, and in butter, the survival never exceeded 2 weeks. On straw, flour, and cow-hair, the virus persisted for from 5 days to 7 weeks. Hay and bran were found capable of promoting the survival of virus dried on them for 15 and 20 weeks, and in this respect they stand out very distinctly from all the other materials tested (5). Some of these results are shown in tabular form in Table IV, in which is included an observation by Trautwein (1926 (6)) on the survival on stable-dust.

Table IV.

Material	Maximum survival	Material	Maximum survival
Glass	10 days	Wool	2 weeks
Sand	14 ,,	Cow-hair	4 ,,
Stable-dust	11 ,,	Hay	15 ,,
Paper	2 ,,	Bran	20 ,,

An explanation of this preservation on hay and bran has not been found, though it has been observed that clear, filtered extracts from these food-stuffs are almost equally effective. It should be noted, however, that this prolonged survival only occurred under conditions involving relative dryness, and absence of light.

For instance, when contaminated hay and bran were kept in an atmosphere saturated with moisture, the virus was inactive after 5 days; and when they were allowed to remain exposed to light, at the temperature and water-vapour pressure of the room, the virus was dead in 3 weeks. Yet, these conditions having been satisfied, the survival of the virus on hay and bran was regularly prolonged to 8 weeks and more. It seems probable, therefore, that in dry, cool and dark, natural surroundings the virus may persist on some kinds of cattle-fodder for some months.

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V. DEMONSTRATION OF THE LESIONS OF FOOT AND MOUTH DISEASE IN GUINEA-PIGS.

By I. A. Galloway, B.Sc., M.R.C.V.S.
(*National Institute of Medical Research, Hampstead.*)

Mr Galloway showed a number of specimens demonstrating the naked eye lesions in cattle, sheep and pigs produced by the virus of foot and mouth disease.

In cattle, vesicles may be seen on the tongue, the inner surface of the lips, on the gums, on the hard palate, as well as on the inner surface of the cheeks. These vesicles vary in size from that of a pea to an egg and contain a clear watery colourless or yellowish fluid, which later becomes cloudy. Later the vesicles burst, the contents escape and the epithelial covering being thrown off, reddened moist, painful flat erosions remain. Vesicles may also be met with on the feet and on the teats of cows.

As a result of the foot infection it may frequently be observed that the coronary seam becomes separated from the sensitive laminae and later sloughing of the hoof occurs.

In pigs, lesions are most commonly met with on the snout and on the feet. In sheep lesions are quite frequently met with only on the feet although mouth lesions also occur.

The specimens showed vesicles and ulceration on the tongue and hard palate of cattle, ulceration of the hard palate of sheep, ulcers on a pig's snout, ulceration of cow's teats after rupture of the vesicles, and separation of the hoof from the sensitive laminae in a pig's foot. One specimen was of particular interest since it showed a pig's foot with two growths of horn resulting from a double infection with two strains of foot and mouth disease. The pig from which the foot was taken had been infected primarily with a strain (Vallée *A*) and 5 weeks later with another strain (Vallée *O*)—the fact that no immunity had been conferred by the first infection to the second confirmed other experiments with regard to the plurality of strains of the virus of foot and mouth disease.

In addition, specimens were shown which demonstrated the primary vesicles on the metatarsal pads of guinea-pigs infected experimentally, and secondary vesicles on the tongue and fore pads after generalisation of the virus.

A rabbit's tongue was shown with ulceration following upon experimental foot and mouth disease infection. As explained by Mr Galloway, in experiments carried out in conjunction with Dr Nicolau no lesions had been observed in rabbits infected either by direct inoculation of the virus into the mucous membrane of the tongue, or by the intravenous route, other than in the mouth.

Mr Galloway then presented a series of lantern slides prepared from microphotographs showing in a complete detailed manner the histogenesis of the lesions appearing on the tongue of experimentally infected guinea-pigs and rabbits from the earliest modification of the epithelium up to the final stage.

The work of previous investigators was referred to including that of Gins who had spoken of "specific" inclusions in foot and mouth disease infection which according to the findings of Trautwein can also be demonstrated in the internal organs such as the stomach, pancreas, duodenum, and spleen and which apparently can be produced by various irritants (heat, acid, etc.).

The present study had been undertaken to determine definitely if tissues other than the epithelium participated in the formation of the lesion, to see whether other tissues were attacked previously or simultaneously with the epithelial tissue and finally to establish whether the affinity of the virus was specific for the epithelial system and for that alone. The general conclusions were that the initial lesion takes place in the epithelial tissue, a degeneration of the cytoplasm of the cells follows. Owing to this degeneration the cytoplasm becomes acidophilic. A type of degeneration of the nucleus which is commonly met with is that termed "chromatin block"—condensation of the chromatin. A complete study of this nuclear degeneration had shown that it takes place as follows: the chromatin which is normally dispersed in the nuclear mass begins by forming unequal particles which approach the centrosome. In a later stage many of these agglomerations fuse with the nucleolus. Finally, all the karyoplasm fuses together to form in the interior of the cell a single mass irregular in form, intensely chromatophilic, greatly reduced in volume as compared with the normal nucleus and not presenting any definite structure.

Another type of nuclear degeneration sometimes seen is that where the chromatin condenses towards the periphery of the karyoplasm and becomes attached to the nuclear membrane.

A destruction of the altered cells occurs and infiltration with polymorphonuclear leucocytes accompanies the degenerative process. The appearances suggested a true intra-epithelial culture.

Degenerated and infiltrating cells are subsequently fragmented and the initial lesion becomes successively a vacuole, a vesicle, and vesico-pustule, followed later by rupture, ulceration and "crust" formation. The participation of the corium is accidental and secondary.

A complete anatomo-pathological study of the organs (liver, brain, spleen, kidney, spinal cord, lung, heart muscle, ovary, testicle, adrenal and parotid) of infected guinea-pigs and rabbits had shown that they were entirely devoid of specific changes.

Mr Galloway referred briefly to experiments in collaboration with Dr Nicolau carried out concurrently with those mentioned above, with regard to the distribution and localisation of the virus of foot and mouth disease in the organism of infected rabbits and guinea-pigs. These experiments had shown that the sole tissue which lent itself perfectly to the seeding, survival, and culture of the virus *in vivo* was the buccal epithelium and pad epithelium in the case of guinea-pigs, and the buccal epithelium in the case of rabbits.

After the 3rd day in guinea-pigs the virus disappeared from the blood and the internal organs and could only be located in the tongue and pads up to the 8th day after infection. In the case of the rabbit, the virus disappeared from the blood and the various internal organs on the 2nd day after infection and could only be recovered later from the buccal epithelium where it was found up to the 5th day. The virus was searched for in the blood, spleen, tongue and buccal epithelium, parotid gland, mesenteric gland, pad epithelium, bone marrow, ovary or testicle, brain, lung, kidney, liver, adrenal gland, thymus, and spinal cord, but was only found in those sites referred to above.

REVIEWS

Principles of Soil Microbiology. By Selman A. Waksman. Pp. xxviii + 897. London: Bailliere, Tindall and Cox. 1927. 45*s*. net.

This volume is worthy its dedication to Professors Beijerinck and Winogradsky for it stands out markedly in the somewhat monotonous landscape of soil science. To give an idea of the general nature of the work one cannot do better than quote from the author's preface.

"An attempt has been made to compile a book which will be of service not only to the investigators in soil science, but also to workers in allied sciences, especially botany, plant physiology, plant pathology and bacteriology, as well as to the general student in agriculture."

"This book is a collection of known facts concerning micro-organisms found in the soil and their activities; it is a study of the literature dealing with the science in question; it is an interpretation of the facts already presented; it indicates the various lines of investigation and notes where further information is especially wanted. Soil microbiology is a science which is at the very base of our understanding of agricultural processes and the practice of agriculture; it comprises a number of sciences. The book may, therefore, be looked upon more as an introduction to further research rather than as an ordinary text-book; as of help to those working in the allied sciences, who are desirous of obtaining some information concerning the soil population and its activities."

A brief analysis of its contents will show the magnitude of the work. The book is divided into four parts. Part "A," which consists of one chapter containing fifty-three pages, is devoted to a consideration of the numbers of different groups of micro-organisms found in the soil and the quantitative methods of their study.

Part "B," which occupies a little less than half the book, contains thirteen chapters dealing with the isolation, identification and cultivation of soil micro-organisms and it is of interest to note the relative space accorded to the several groups. Eight chapters containing one hundred and sixty-two pages are given to the bacteria; one chapter of twenty-one pages to the algae; one chapter of forty-nine pages to the fungi; one chapter of twenty-six pages to the actinomycetes; one chapter of thirty pages to the protozoa and one chapter of twenty-six pages to the non-protozoan fauna comprising flatworms, nematoda, rotatoria, annelida, tartigrada, arthropoda, arachnida, myriapoda, insecta and mollusca.

Part "C," which comprises rather less than a third of the volume, deals with the chemical activities of the soil micro-organisms. An introductory chapter of seventeen pages, treating of the general principles of microbial metabolism, is followed by two chapters containing eighty-one pages in which are discussed questions of energy transformations in the metabolism of micro-organisms and the chemistry of the decomposition of non-nitrogenous organic matter in the soil. There are then five chapters comprising one hundred and thirty pages dealing with various aspects of the relation of the soil population to the nitrogen cycle. In a final chapter of nineteen pages is discussed the transformation of sulphur by micro-organisms.

Part "D," about one-third of the volume, is concerned with soil microbiological processes in relation to soil fertility. An introductory chapter of twenty-five pages is devoted to a consideration of the soil as a medium for the growth and activities of micro-organisms. There follow two chapters containing sixty-four pages in which an account is given of the transformation of minerals and organic matter in the soil. In a chapter of thirty pages is then discussed the question of the microbiological analysis of soil as an index of soil fertility and this is followed by two chapters

comprising sixty-three pages in which is considered the influence of environmental conditions upon the microbiological equilibrium in the soil and upon the activities of the several groups of organisms. A chapter of sixteen pages follows in which the soil is considered as a habitat for micro-organisms causing plant and animal diseases and a further chapter of seventeen pages discusses problems of soil inoculation. A concluding chapter of ten pages treats of the history of soil microbiology, its past, present and future.

In addition there is a classified list of over two hundred books for reference in soil microbiology, eight pages of contents, twenty-one pages of author index, thirty-one pages of subject index, nineteen plates of illustrations, seventy-seven text-figures and ninety-four primary tables. References are put as footnotes and these make available a wealth of literature often from Russian and other out of the way sources.

A book of this nature, a soil Baedecker nine hundred pages long, obviously cannot be "reviewed" in the ordinary sense: one can only accept it as a somewhat Brobdingnagian guide to a world of Lilliput and wonder at the encyclopaedic and industrious quality of the author's mind. This wonder is increased when one remembers the torrent of experimental papers that has poured from the author's pen during the last decade—in the index of authors, references to Beijerinck number forty-six, to Winogradsky thirty-eight, to Waksman sixty-nine—that last winter he and Davison produced a book of over three hundred and sixty pages on "Enzymes," that in the spring he contributed extensively to Abderhalden's *Handbuch*, that he is an Associate Professor of Soil Microbiology at Rutgers University and Microbiologist of the New Jersey Agricultural Experimental Stations and that in his spare time he acts as secretary for International Conferences and travels round the world. He probably does many other things and one marvels how a worker of such amazing productivity contrives to live on twenty-four hours a day unless in possession of Well's time machine or the secret of perpetual motion.

There is probably no other soil investigator whose personal researches have ranged more widely in so many different fields and this experience is shown to advantage in the present volume. Further, treatises of this order are usually old fashioned by the time they are born but, in the present volume, this is not the case, for owing to the amazing speed at which the author works a great deal of 1926 matter is incorporated in the actual structure of the book.

The work is, of course, not impeccable for in these days of intensive specialism no one can keep up to date in a dozen different pastures of biology and chemistry and show throughout an equal degree of critical acumen and scientific vision or write with equal authority. In my hands, for example, the book chanced to open first at page 237 and in the commencing paragraph "*Synchitrium endobioticum*" is referred to as a myxomycete and over the page my own name is misspelled. In reading through the volume such errors occur not infrequently yet, in consideration of the magnitude of the work, they are trivial matters to be amended in a second edition. There are also, as could only be expected in a treatise of this nature, frequent occasions when one seriously differs from the author in his interpretation of data or the balance of emphasis adopted in his treatment of relative values. The author's judgment must, of course, stand or fall by the pragmatic test of fitness to new data and alignment with the future trend of the science, but the present differences of opinion make the volume all the more stimulating.

The book has appeared at a very opportune moment, for the time was ripe for its production. For half a century an increasing amount of attention has been devoted to the soil population; at first only to the bacteria but more recently to the other groups of organisms and the balance of our knowledge to-day is reflected in the relative numbers of pages given to the several aspects in this volume. The bacteria still receive the lion's share of attention partly because they were "the first in the field" and partly because they are the easiest to study, morphological considerations being practically eliminated. This period of bacterial dominance is now quite clearly coming to an end. The importance of the protozoa and the algae has been demonstrated and the quite outstanding importance of the soil fungi is being increasingly recognised. Investigations on the latter group, meagre though these have been, have given results of such promise that the fungi bid fair to outrival

even the bacteria in the attention they demand. There can be little doubt that were the fungi studied with the same degree of intensive application that has been given to the bacteria this group would assume an altogether different status in our ideas of the relative importance of the several groups of micro-organisms in the soil economy. The author's cross-section of soil microbiology has appeared just at the time when a saner and more balanced view-point is becoming apparent in the science and it is valuable to have this work to mark the end of an early and rather immature period.

We are at the close of a period in another way. During the last half century research on soil microbiology has been entirely analytic, a taking to pieces of the soil system and an examination of the living fractions under test conditions in the laboratory. Synthesis remains almost a virgin field and yet, obviously the larger problem, in fact the real problem confronting us is to discover how the fractions relate to each other, how the parts interlock to make a system that is at once a congeries of parts and yet a unity; a self regulating organisation. Analysis is easy compared with synthesis. Synthetic research demands that in our mental imagery the analytic phenomena be perceived in a kind of relational panorama or mosaic; it demands a reversal of our common ways of thought and an abandonment of the preconceived notions and scientific assumptions drilled into us in our almost purely analytical training. In soil microbiology we are beginning to realise the urgent need for a more synthetic approach to our problems and it is as a prepared survey enabling one to choose a vantage ground for jumping off into this unknown that the present volume possesses much of its value.

It is greatly to be hoped that Waksman's *Principles* will find its way into the academic schools of botany and zoology and the schools of medicine and not be valued only by those interested in agriculture. There can be few primary fields of biological research that have been more neglected in the university schools than the study of the population of the soil. Attention has been so concentrated on the fauna and flora of the surface of the soil and of aquatic habitats that the underworld of life has been almost passed by, and yet the study of the soil and its population is clearly fundamental to an understanding of the life and ways of higher forms. Also, soil microbiology contains endless problems of research such as could very suitably be carried out in academic laboratories and a little closer contact with the soil would not do any botanist or zoologist any harm.

In concluding one would say that in writing *Principles of Soil Microbiology* Professor Waksman has performed a notable task; one that has placed his name in the front rank of those who are writing biological books to-day. Soil microbiology will largely date before or after Waksman's *Principles.*

WILLIAM B. BRIERLEY.

The Microbiology of Cellulose, Hemicelluloses, Pectin and Gums. By A. C. THAYSEN and H. J. BUNKER. Pp. vii + 363; 23 Figs. Oxford University Press. 1927. 25*s.* net.

The authors' aim as stated in the preface to this volume is to give "a comprehensive account of the information available" on the "microbiological destruction of cellulose" and on the "allied subjects of the microbiological changes occurring in hemicelluloses, pectin and gums." "The treatise has been compiled from the point of view of the research worker who desires to know in what direction his efforts may most profitably be directed within this important subject of the natural and artificial decomposition of vegetable tissues." This is a large and difficult task and the authors, whose original researches have contributed materially to our understanding of certain aspects of these problems, have achieved a large although perhaps not a complete measure of success in their attempt.

The book is divided into four parts of which the first is introductory containing two short chapters on the occurrence and properties of cellulose, hemicellulose, pectin and gums and on the importance of the microbiological aspect of their study.

Part II deals with the types of micro-organisms associated with the decomposition of these substances. Chapter III gives in some detail an account of those members of the Eubacteriales which gain their energy from these compounds, an undue amount of space perhaps being devoted to the *Spirochaete cytophaga*. Chapter IV deals somewhat briefly with the Actinomycetes. Chapters V and VI, which together constitute about a quarter of the book, contain descriptions, taken almost entirely from Rabenhorst, of those Eumycetes which have been recorded as decomposing pectin and hemicelluloses or cellulose and lignin. This extensive treatment would seem unnecessary, for any practical student wishing to identify his fungi must go either to the special systematic treatises or to the taxonomist—he cannot use this volume for diagnosis or as a systematic work. The most required here, perhaps, was a list of fungi with references, but the value of even such a list seems doubtful for the ability to decompose these substances is so common among the fungi that workers rarely make special record of its occurrence. In these two chapters the authors are obviously writing with little personal experience of this portion of their subject.

Part III treats of the microbiological decomposition processes of gums, pectin, hemicelluloses and cellulose. Chapter VII deals with the retting of fibre plants through the decomposition of gums and pectin. Chapter VIII discusses the resolution of hemicelluloses and cellulose especially in the manure and compost heap, in the soil, under water and in the intestinal tracts of animals. The authors' own researches have lain primarily in the fields of applied bacteriology and the book tends, in consequence, to emphasize the essential importance of the bacteria. In view of the more recent work of Rege and others it seems possible that opinions concerning the relative importance of fungi and bacteria in these processes may need revision. Chapter IX discusses in a comparative manner the formation of silage, the spontaneous heating of straw and the fermentation of tobacco, cocoa and coffee. In Chapter XI is given an account of micro-organismal attack upon cellulose-fibres and fabrics, fishing nets and so forth, special attention being devoted to the very useful swelling test devised by the authors. Chapter XII deals with the destructive action of fungi on lumber, manufactured wood and wood pulp.

Part IV contains a single short chapter indicating certain of the applications of microbiological reactions to the manufacture of industrial compounds, such as combustible gases, power alcohol and organic acids from hemicelluloses and cellulose.

Each chapter is followed by a list of references which, in view of the authors' stated aim of giving "at least a broad outline of the more essential literature already in existence" show unexpected omissions. There is a useful index of authors and a good subject index, a great desideratum in a book of this nature. The work is illustrated by a few text-figures and nine plates, some of which might be regarded, perhaps, as unnecessary.

The book will be found very useful, for since Lafar's *Handbuch der technischen Mykologie* there has been no general treatise on this subject, but it cannot be regarded as a complete success or as really filling the gap. The authors have tried to cover too wide a field. The first half of the volume dealing systematically with the micro-organisms concerned, and especially that portion dealing with the fungi, is too incomplete to be of permanent value: a complete treatise on this aspect would run to several volumes and we already possess our systematic compendiums. The second half, containing an account of the decomposition processes in their industrial applications is by far the better portion of the book, although even this gives, perhaps, the impression of being written somewhat hastily and with not sufficient consideration. Even so, had it been enlarged to fill the whole volume the book would have been a greater success, for a full treatise on the more modern aspects of these problems is badly needed.

The authors have, however, in this work made a very courageous attempt and their partial failure is largely due to the sheer size of the problems attacked and the limited scope of a single volume. It is extremely doubtful whether it is in any way possible to combine in one volume adequate treatments, both of the systematology of such vast groups as the fungi and bacteria and, also, of the physiological and biochemical activities of these organisms even in only a specific field of their

industrial relationships. Systematic diagnosis, in particular, is a thing by itself, mostly useless or vicious in practice unless based on relatively complete keys. It would seem out of place in such a book as this and had far better be left to special treatises.

WILLIAM B. BRIERLEY.

Standard Methods of the Division of Laboratories and Research of the New York State Department of Health. By A. P. WADSWORTH. Roy. 8vo. Pp. xx + 704; 12 Plates. London: Bailliere, Tindall and Cox. 1927. 34*s*.

The central laboratory of the New York State Department of Health is recognised as one of the best managed and most efficient public health laboratories in the world. Its activities include the examination of water, sewage, industrial waste-products, milk, ice, the diagnosis in suspected material of diseases such as rabies, syphilis, tubercle, pneumonia, etc., the preparation and standardisation of antitoxins, sera, vaccines (including tuberculin), and the thousand and one services which fall within the scope of every health department. Reorganised in 1914 as a branch of a large department of the State Government, it has grown continuously, developing its contact with the smaller laboratories outside the Greater City of New York until now there are 105 approved laboratories serving the State, all in close contact with, and to some extent under the supervision of, the central laboratory. Out of this development has arisen the necessity of standardising the methods in use throughout the State, and in this volume Dr Wadsworth gives an account of the routine practice in every section, with the threefold object of keeping uniform the established methods, of instructing the new worker, and of finding the policy which the trained and responsible worker shall adopt, or depart from, as the immediate situation may demand. The account is detailed and exact, and ranges from the broad principles regulating the relations of the laboratory to other laboratories and the public to such minutiae as the cleansing of glass-ware, the labelling of slides and preparations, the filing of records, as well as the management of the library and the technical processes adopted in each of the many forms of its activity; and as it is the product of a very large and remarkably organised experience, the methods described can claim to have a value which has been confirmed by the test of extensive practice. Naturally the scope of the work undertaken and the equipment for carrying it out are on such a scale that many of the methods described and the procedures recommended are far beyond the capacity of most laboratories in this or any other country, and the exposition of the organisation of the various sections can be of direct significance only to large municipal authorities, or large areas combining to maintain a central institution; but even for smaller units the assemblage into one volume of a mass of formulae, receipts, procedures (*e.g.* the making of media, or stains, the care of instruments, the keeping of animals, and the like), with many of the small practical hints that mean so much to success, makes this book a convenient work of reference, whose value is enhanced by an index commendably full.

J. HENDERSON SMITH.

Schädlingsbekämpfung. Grundlagen und Methoden in Pflanzenschutz. VON DR WALTHER TRAPPMANN. Pp. 440. Leipzig: S. Hirzel. 1927. R.M. 20.

The study of methods for the control of pests and diseases of cultivated plants is a branch of applied science with almost equally important physical, chemical and biological aspects; and the literature is widely scattered in scientific journals of all kinds. There is, therefore, special need for clear presentation of the subject as a whole; but this has been seldom attempted, and until the appearance of the volume under review, Wardle and Buckle's admirable *Principles of Insect Control* has stood almost

alone. To a considerable extent, Dr Trappmann's book covers much the same ground, but the scope is wider by the inclusion of pests other than insects and the whole subject is approached from a somewhat different angle. As the sub-title indicates, the book deals with general principles, and special cases are only referred to by way of examples. There is no division of subject-matter as between insect and fungus pests.

The author has arranged his material in a clear and logical manner; and reference is readily made to any particular point. After some introductory sections on plant diseases, on the relations between plants and their parasites, and on the occurrence and distribution of pests, general methods of control are dealt with under four main headings: cultural measures; biological control; control by physical means (traps, grease-banding, etc.); and control by chemical means. The last forms the main part of the book and in regard to plant pests is a fairly full account. Pests of livestock are not discussed. Matters which receive specially full treatment are the physical properties of spray-fluids, spraying and dusting machinery, fumigants and "seed-pickling," the latter including an account of the newer German fungicides containing mercury compounds.

The large subject of biological control is discussed comparatively briefly. The author regards it as "ein gutes Hilfsmittel," but with limited possibilities because success is dependent on many factors not under control. Cultural methods, such as the breeding of immune varieties of plants, possess the utmost importance for the future; but for the present, control by physical and chemical means must take the first place, and will probably always be "a necessary evil."

An interesting section is devoted to the discussion of methods for testing insecticides and fungicides, the experimental conditions essential for success in evaluating new materials by biological tests both in the laboratory and in the field being fully explained and the limitations of the methods indicated. The author holds that the information given by biological tests under laboratory conditions is of great importance for insecticides, but of little value for fungicides (except in the case of materials for seed treatment), because of numerous external conditions affecting the incidence of fungus attack which are almost impossible to imitate in the laboratory. He considers that fungicides must be judged almost entirely by their performance in the field, perhaps another way of saying that satisfactory laboratory methods remain to be worked out.

It is striking to note that in dealing with different types of insecticides and fungicides, the author is obliged to admit, in almost every case, that the manner in which toxic action is exerted is not yet explained. There is great need for more accurate fundamental knowledge on this point, and until this is available further progress with chemical control measures would seem likely to be slow and uncertain.

Few substances find mention which are outside the well-known standard groups of insecticides and fungicides. Dr Trappmann perhaps hardly does justice to the recent work in America on sodium and calcium fluosilicate dusts which he dismisses very briefly. A recent report from the U.S. Chemical Warfare Service discloses further promising results with these materials.

Dr Trappmann has written a book which will be of value to all workers in the field with which he deals. One may, however, be permitted a final grumble. There are references to much of the recent literature; but one cannot fail to remark that English work is almost completely ignored. Are English journals still inaccessible in Germany?

C. T. GIMINGHAM.

The Structure and Development of the Fungi. By H. C. I. GWYNNE-VAUGHAN and B. BARNES. Pp. xvi + 384, with 1 Plate and 285 Text-figs. Cambridge University Press. 1927. 15*s*. net.

Owing to the significance of the fungi in agriculture and industry the publication of a treatise on this group of plants is an event of first class importance. Apart from

the accounts of the fungi in such phytopathological volumes as those of Duggar, Stevens, Harshberger, Butler, Nowell, Heald, etc. the only general treatise on the fungi, in the English language, during the last quarter of a century is the small and unsatisfactory text-book published by Massee in 1906. In addition there have been V. H. Blackman's outline in the *Encyclopedia Britannica*, Gwynne-Vaughan's volume on the *Ascomycetes, Ustilaginales and Uredinales*, short accounts in general text-books of botany, popular works such as those by Swanton, Rolfe, etc., and volumes on the systematy of special groups by Grove, Rea and others. The Continent has produced numerous systematic and phytopathological works, outlines such as those by Fischer, Janke, etc., or more general accounts such as that by Lotsy in his *Vorträge über botanische Stammesgeschichte*. Only one treatise on the fungi *sensu stricta* has appeared, which is Gäumann's *Vergleichende Morphologie der Pilze* published in 1926. The need has been urgent for a work in English on this group and the present volume by Gwynne-Vaughan and Barnes is assured in advance of a cordial welcome.

The immediately noticeable thing about the two recent books is that they are concerned solely with the morphology and largely the cytology of fungus reproduction, the physiology and biology of the fungi being in one case omitted entirely and in the other case almost entirely. This severe morphological attitude is to a large extent pathognomic of the state of academic mycology and can be traced directly to the dominating influence of De Bary's work published in 1884. In his preface to this volume De Bary clearly stated that as the physiology of the fungi had received comprehensive treatment elsewhere he would confine himself to their morphology. Unfortunately De Bary's volume became accepted not at his own evaluation, *i.e.* as a presentation of the morphological aspects only of the fungi to be complemented by equivalent treatment of the physiological and biological aspects, but as a general treatise on this group. The magistral character of the work, the absence of any other volume on the fungi of like calibre, and its translation almost immediately into English caused it to dominate mycological study the world over. In consequence the somewhat smaller but much more balanced *general* treatise, *Die Pilze*, published by Zopf in 1890, was practically overlooked and never translated into English. Had Zopf's work appeared first and been translated and not that of De Bary, mycological study would probably have run a very different course. The acceptance of a partial morphological view-point became confirmed during the era of acute botanical specialisation which set in about that time. The study of the morphology, systematy, physiology and biology of the fungi, which previously had made one subject, began to disintegrate and to follow four distinct and largely divergent paths. The academic mycologist with his De Baryan bible confined himself almost entirely to working out the lines suggested by the "Master" and became a pure morphologist and cytologist. The present condition of this line of development is crystallised in the volumes by Gwynne-Vaughan, Gäumann and by Gwynne-Vaughan and Barnes. The systematic study of the fungi became almost entirely a matter for herbarium specialists in whose hands it still remains and to whom we are indebted for all the major systematic treatises on this group. The study of the physiology of the fungi fell largely into the hands of the biochemists who have developed it primarily in its fermentative and other applied aspects. As the outcome of this avenue there are the relevant parts of the treatises by Lafar, Henneberg, Führmann, Kruse, Jörgensen, Waksman, Thaysen and Bunker, etc. The biology of the fungi became largely the pasture of plant pathologists and its development has resulted in such treatises as those by Sorauer, Butler, Kirchner, Ferraris, Maublanc, Duggar, Fawcett and Lee, Marchal, Petch, Nowell and many others. Naturally there are exceptions to what has been said above but as a general picture it is probably true. The strict morphological outlook of academic mycology is seen if one compares the amount of space allocated in certain scholastic volumes to morphology and to physiology and biology respectively. In "De Bary" 87 per cent. is given to morphology, 13 per cent. to physiology and biology: in "Zopf" the ratio is 66 to 34; in Gwynne-Vaughan's volume of 1922 the ratio is 87 to 13; in the present volume by Gwynne-Vaughan and Barnes 86 to 9 (with an additional 5 per cent. on technique of study) and in "Gäumann" the entire work is morphological. It is clear that for any student to obtain a balanced and comprehensive view of the fungi the volumes

by Gäumann or by Gwynne-Vaughan and Barnes need to be complemented by some physiological treatise such as that by Kruse or the relevant portions of Lafar and by more biological works such as Buller's *Researches* together with some phytopathological volume such as that by Höstermann and Noack, Duggar, Butler or other plant pathologist. The ideal volume on the fungi has yet to be written, but it clearly would be something akin to Lorrain Smith's treatise on the Lichens published in 1921 in which about 5 per cent. of the space is allocated to history, and the remainder of the volume divided more or less equally between morphology and classification on the one hand and physiology and biology on the other.

What has been said above is in no sense a direct criticism of the present volume for the title of this is *The Structure and Development of the Fungi*, and it makes no pretence of being a general treatise on this group: it is purely and simply a morphological work with a general introduction. The danger is that, like De Bary's volume of forty years ago, it may be accepted by academic students and teachers as a *general* text-book and so help to continue the present unbalanced perspective in which the more physiological and biological aspects are divorced from the morphological aspects which are the essential mycology of the academic schools of botany.

The volume itself consists of some 40 pages of general physiological and biological introduction followed by 278 pages of comparative review of the systematy of the fungi and the cytology of their reproductive processes, 20 pages devoted to mycological technique, 30 pages of bibliography and an Index. The book is very well illustrated containing one plate and 285 text-figures, many of which are original, but from a considerable number of which any statement of magnification is omitted.

In reading through the volume, and more especially in the introductory section, one finds occasional loose wording which might lead to ambiguity, but on the whole the book is singularly free from errors and misprints. There are of course points of detail at which one might cavil; questions of life-histories such as whether the conidial and perithecial forms included in *Theilavia basicola* do not really belong to different fungi; questions of interpretation such as whether the nutritive hypothesis of heterothallism favoured by the authors is in any way acceptable or consonant with the evidence; questions of relative values such as whether the list of culture media on page 333 does not omit many of the most widely known and useful media, and so forth.

It is difficult without entering into considerable detail to discuss the main theme of the volume, but it is interesting to compare the systematic arrangement adopted by the authors with the arrangements in Gäumann's volume and in Wettstein's *Handbuch der Systematischen Botanik* (3rd edit. 1923). Wettstein divides the Phycomycetes into four orders: (1) Chytridiales, (2) Monoblepharidales, (3) Oomycetes (with sub-orders—Saprolegniineae, Ancylistidineae, Peronosporineae) and (4) Zygomycetes. Gwynne-Vaughan and Barnes divide the Phycomycetes into three orders: (1) Archimycetes (with alliances—Chytridiales, Ancylistales, Protomycetales), (2) Oomycetes (with alliances—Monoblepharidales, Saprolegniales, Peronosporales) and (3) Zygomycetes (with alliances—Mucorales, Entomophthorales). Gäumann separates off the Archimycetes as a Class equal in value to the Phycomycetes, Ascomycetes or Basidiomycetes. In the Archimycetes he includes the families Olpidiaceae, Synchytriaceae, Plasmodiophoraceae and Woroninaceae. In Gwynne-Vaughan and Barnes' treatment the Plasmodiophoraceae are regarded as outside the true fungi whilst the other three families are included in their alliance Chytridiales. Gäumann's Phycomycetes include the three orders Chytridiales, Oomycetes and Zygomycetes.

The Ascomycetes are divided by Gwynne-Vaughan and Barnes into the three orders: (1) Plectomycetes (with alliances—Plectascales, Erysiphales, and Exoascales), (2) Discomycetes (with alliances—Pezizales, Helvellales, Tuberales, Phacidiales and Hysteriales) and (3) Pyrenomycetes (with alliances—Hypocreales, Dothidiales, Sphaeriales and Laboulbeniales). Wettstein separates off the Endomycetaceae and Saccharomycetaceae, which are included in Gwynne-Vaughan and Barnes' Plectascales, as a first group the Protoasci, putting all the other Ascomycetes in a second group the Euasci. This latter contains seven orders: (1) Perisporiales, (2) Plectascales, (3) Discomycetes (with four sub-orders—Hysteriineae, Phacidiineae, Pezizineae, Helvellineae), (4) Tuberales, (5) Exoascales, (6) Pyrenomycetes (with sub-orders—

Hypocreineae, Dothideineae, Sphaereineae), (7) Laboulbeniales. Gäumann also divides the Ascomycetes into two classes, the Hemiasci or Protoasci and the Euascomycetes, but in the former places the two orders (1) Endomycetales (with families—Dipodascaceae, Endomycetaceae and Saccharomycetaceae) and (2) Exoascales (with families—Protomycetaceae and Exoascaceae). The Euascomycetes are then divided into twelve orders: (1) Plectascales, (2) Perisporiales, (3) Myriangiales, (4) Hypocreales, (5) Sphaeriales, (6) Dothidiales, (7) Hysteriales, (8) Hemisphaeriales, (9) Phacidiales, (10) Pezizales (with categories—Inoperculatae and Operculatae), (11) Tuberales and (12) Laboulbeniales. Wettstein divides the Basidiomycetes into three groups: (1) Hemibasidii (with orders—Ustilaginales and Uredinales), (2) Protobasidii (with orders—Auriculariales and Tremellales) and (3) Autobasidii (with orders—Dacryomycetales, Tulasnellales, Hymenomycetes, Exobasidiales and Gasteromycetes which latter are sub-divided into Plectobasidii and Eugasteromycetes). Gwynne-Vaughan and Barnes divide the Basidiomycetes into three sub-classes: (1) Hemibasidiomycetes (with alliance—Ustilaginales), (2) Protobasidiomycetes (with alliances—Uredinales, Auriculariales and Tremellales) and (3) Autobasidiomycetes (with alliances—Hymenomycetales and Gasteromycetales). Gäumann divides the Basidiomycetes into two sub-classes, the Protobasidiomycetes and the Autobasidiomycetes. The former contains four orders (1) Auriculariales, (2) Uredinales, (3) Ustilaginales and (4) Tremellales, whilst the Autobasidiomycetes contain seven orders: (1) Tulasnellales, (2) Dacryomycetales, (3) Cantharellales, (4) Polyporales, (5) Agaricales, (6) Plectobasidiales and (7) Gastromycetes. In all three volumes the fungi Imperfecti are merely noted as a hotch-potch at the end. Thus, throughout, the arrangement of Gwynne-Vaughan and Barnes is more simple than that of Wettstein and greatly more so than that of Gäumann. Simplicity is without doubt meritorious, but whether it always gives the truer picture is a matter for considerable question. In any case general systematic arrangements of the fungi at present are bound to be very tentative and to be strongly biassed by the author's personal predilections and, under these conditions, the simplest possible arrangement consonant with the data has many advantages. The clear and straightforward way in which the authors have worked through their systematic treatment and laid it out for inspection is in itself most praiseworthy. Whether their particular arrangement will stand is entirely a matter for pragmatic test, but it certainly appears to be a workable and logical formulation.

It is quite unnecessary to recommend this volume for it is the only one available for English speaking students of the fungi and it partially fills a gap that has existed for a quarter of a century. The book is excellently produced and is reasonable in price and will at once become the standard text for University and other students of the fungi. Providing students realise that this volume only partially covers the mycological field and needs supplementing on two other equally large aspects, they cannot have a better guide to the study of the systematic arrangement of the fungi and the cytology of their reproductive processes.

WILLIAM B. BRIERLEY.

Agricultural Parasitology. By C. L. WALTON and W. REES WRIGHT. Pp. vi + 122, with 6 Plates and 16 Text-figs. London: Sidgwick and Jackson, Ltd., 44, Museum Street, W.C. 6*s*. net.

The aim of the authors of this book has been to produce a handy volume dealing with the salient facts about the animal parasites of farm stock in the British Isles, suitable for students taking a course in agricultural zoology.

In arrangement and style the work suggests a series of lecture notes, and in fact it is based on a course of lectures given for some years by the senior author at University College, Bangor.

It consists of twelve chapters and an appendix, the latter being devoted to a brief introductory account of the principles of classification and nomenclature, in zoology. In Chapter I the elementary features of the various aspects of parasitism are explained. Chapter II deals with the parasitic Protozoa. Of the remaining

chapters, three are devoted to parasitic worms (III–V), one to mites and ticks (VI), five to insect parasites of farm stock (VII–XI) and in the final chapter instructions are given regarding the collection and preservation of parasites. A few references are given at the end of each chapter, the majority being to comparatively recent publications. The Plates are good but some of the text-figures (*e.g.* 14 *D*) are too sketchy to be of real help to the student.

One has the feeling after reading through this book that the authors have endeavoured to produce a small volume which could be sold at a modest price. In these days of many text-books this is a great consideration for the student, but we think that the usefulness of the book would be enhanced if in a future edition certain of the sections were extended and some good text-figures introduced. A list of the parasites dealt with, arranged under hosts, would also be a useful guide.

It is evident, particularly in certain sections (*e.g.* Chapters III, Trematodes; X, Diptera), that the authors have had considerable field experience and remedial measures where recommended are in general practical and clearly stated. On the whole, however, the book gives the reviewer the impression of having been hurriedly prepared and some of the errors we have noted, considering its modest size, should have hardly escaped detection. The meaning of a few sentences (*e.g.* p. 50, line 23; p. 51, line 34; p. 102, line 9) is not clear. *E. steidai* (footnote, p. 15) presumably should read *E. stiedai* to agree with the spelling on line 5. *Myasis* (p. 73) we think should read *myiasis.* In the statement "the remaining pairs of *tracheae* in the bee," etc.... (p. 55) probably *spiracles* is meant. In the legend of fig. 9 *tracheae* should read *trachea.* In the section on fleas (p. 101) the emergence of the adult from the cocoon is apparently referred to as "an interesting feature connected with hatching." The abbreviation "cms." is inadvertently used for centimetres (pp. 20 and 32) since "cm." is used elsewhere and mm. is used for millimetres.

The information contained in the book is up-to-date and the agricultural student will find it useful as an aid to refreshing his memory regarding the essential facts about the animal parasites of farm stock in the British Isles.

J. DAVIDSON.